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RESEARCH PAPER

Bioremediation of Oily Wastewater by Using of Bacteria (*Bacillus subtilis)*

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A B S T R A C T:

This paper is trying to arrange to develop the efficiency of the wastewater treatment system of a petroleum refinery (Namely KAR Refinery) by using bacterial (Bacillus subtile) bioremediation treatment. Wastewater samples have been collected from system output from October 2018 to March 2019. After the collection of the sample, the author treated the samples by adding different weights of powder bacteria (5,10, and 15gm) to 10 L of wastewater samples. Oily wastewater samples were examined before and afterward treatment for phosphate (PO_4), total hardness, ammonia (NH_4), chloride (CL^{-1}), and analysis for hydrocarbons by using GC-MS. The results indicated the effectiveness of 15 gm of powder bacteria's best use of wastewater bioremediation technique caused a decrease in the value of hydrocarbon affectedly.

Keywords: bioremediation, degradation, bacteria, hydrocarbons, oil wastewater.
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1. INTRODUCTION

Crude oil has been defined as an extremely mixture of hydrocarbons, paraffin, and aliphatic compounds with oxygen, nitrogen, compounds containing variable amounts of sulfur and other substances including organic and inorganic minerals (Acuna-Arguelles *et al.*,2003). Crude oil consists of a group of hydrocarbon compounds that are chemically distinct and necessitate reactive mechanisms for initiation and consumption (Arafa, 2003).

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Beside fact of the negative side of petroleum caused yet oil still is an important factor in all the sectors of any country's economy input for sustainable development of the country. However. Those side effects from the process of refinement oil should not be ignored (Thabit, T.H. and Jasim, Y.A., 2016).

Oil has many disadvantages; such as Refining petroleum generates air contamination. Transforming crude oil into petrochemicals discharges toxins into the atmosphere that are dangerous for ecosystem and human health. Burning gasoline releases CO2 because of not complete combustion of oil during refining process (Bhargava, 2017).

Oil contamination can have a harmful effect on the water environment; it extents over the surface in a thin layer that stops oxygen reaching to animals and plants that live in the water. Oil pollution avoids photosynthesis in plants and disturbs the food chain (Enujiugha, 2004).

Wastewaters one of the environmental harms through crude oil-processing and petrochemical industries since the presence of large amounts of crude oil products, polycyclic and aromatic hydrocarbons, phenols, metal derivatives, surface-active substances, sulfides, naphthenic acids and other chemicals (Suleimanov, 1995).

According to Beg, Al-Muzaini , 2003 the discharged wastewaters become acutely threatening, to the accumulation of toxic products in receipt of water bodies with potentially severe significances on the environment. These discharges contain different chemicals at different attentions, including sulfides, hydrocarbons, ammonia, phenol, and water. Other reports have shown a positive connection between pollutants from refinery effluent wastewater and the health of aquatic organisms. Former explanations done by Kuehn et al. (1995) who submitted a relationship between water contamination and sediments with aromatic hydrocarbons from refinery effluents, these big amounts of a chemical substance that appear with the oil will have a toxicity effect on the environment, thus, to protect the environment from the wastewater effect, it must be treating and reusing it for irrigation and industrial use (Aziz, S.Q., Saleh, S.M. and Omar, I.A., 2019).

Furthermore, refinery wastewaters are subjected to different physical, chemical, and biological treatment processes that considerably decrease total emissions, and they are also probable cause to adverse effects on our environment (ECETOC,2019). Numerous scientists have identified different types of organisms that have the potential to consume active hydrocarbons in a natural environment such as *Marinobacter, Pseudomonas, Alcanivorax, Sphinogomonas, Micrococcus, Gordonia Cellumonas.* In addition to fungi, yeasts, and algae (Atlas, 2005 and Collee, *et al.*, 1996).

The procedure of biological treatment is can be seen as one of the best ways to recover water or soil using other living organisms that decompose toxic hydrocarbons. It is a cost-effective and straightforward process that applies to large areas of pollution (Al-Jaff, 1998). Still no researches conducted on Bacteriological treatment of industrial wastewater in the Kurdistan Region.

The aim of this paper to control hydrocarbon in oily wastewater using powder Bacteria with different bacterial count as bioremediation and the physico-chemical properties of wastewater before and after bioremediation.

2.MATERIAL AND METHOD

2.1.Study area

The study area is located in the Khabat area, also known as Kawrkosek, 40 km west of Erbil city and it dominates a land of 2.5km² to the left of the Upper Zab River. KAR locates on 36. 3179° Latitude and 43.7573° Longitude. The Kawrkosek refinery, the fourth largest in Iraq and the most noteworthy private sector, in Kurdistan region of Iraq. The below (Figure 1 and Plate 1 shows the exact location of KAR refinery among the other in green dots). These products such as crude oil, gasoil, benzene, naphthalene, and etc. are stored and distributed in storage tanks and then transported through-loading stations by tankers. The water quality standard of discharged water according to national environment and World Bank, as shown in table (1):

Table (1): water quality standard of discharged water according to national environment and world bank.

Parameter	National environment standard	World bank
Chloride	500 mg/L	250 mg/L
PO4	5 mg/L	2.0 mg/L
Ammonia	10 mg/L	10 mg/L

Total hardness	500 mg/L	_

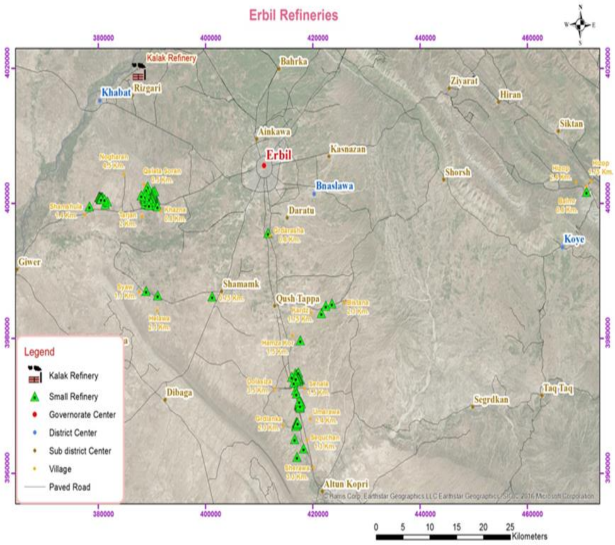

Figure (1): KAR group refinery- khabat (at Kawrgosk village).

Plate (1): discharge of contaminated wastewater after passing the wastewater treatment system (Sampling area).

2.2.Collection of Samples and analysis

Samples were obtained from the discharge point (output) of the treatment system in Kawrgosk refinery- Unit 1, which is the last point of contaminated water treatment after passes through physical, chemical, and biological therapy. This output will discharge to the Kalk River.

The phosphate (PO_4) was determined and defined by APHA (1998), Total hardness measured as $CaCO_3$ by using a test kit HACH, ammonia (NH3) was measured by a portable HANNA device named HI 700, chloride (CL^{-1}) was measured as described in (Bartram and Balance, 1996) and hydrocarbons measured by using GC-MS according to (Marriott, et.al., 2001) before and after treatment.

2.3.Bioremediation model

At the beginning, Broth bacillus bacteria prepared by adding few milligrams of powder bacteria to 10 ml of trypton soya broth media. The broth media incubated 3 – 4 hours at 30 ^{o}C to enrich the growth of Bacteria.

-0.1 ml broth bacteria progress to Petri dish and addition of 20 ml of Trypton Soya Agar on it. After that moving the Petri dishes in infinity shape to make the agar dry and distribution in equals volume, incubated at 18 to 24hours at 37 ^{o}C. Later count the growth colony counts (viable count).

- 2 ml of the growth broth media taken to measure turbidity at 625 nm compared to standard McFarland, 0.1) to count total bacterial cell count.

-After that, from powder Bacteria, the author took different weights (5 gm,10 gm, and 15gm) and added to 10L of oily wastewater at room temperature with aeration using an air pump. The research was carried out with inoculated pools that constituted the control. As shown in Plate (2) and Figure (2) down.

-All pools incubated at 25 ^{o}C for determined hydrocarbon residual for five weeks of adding Bacteria. Residual concentration of crude oil determined by gas chromatography.

-Samples took from oily wastewater analyzed by GC-2014 (SHIMADZU) to determine hydrocarbon depredation compering with oil samples.

Plate (2): preparing pools to full it by oily wastewater

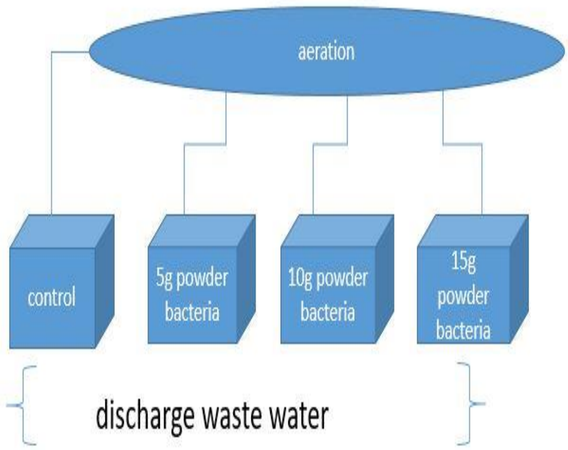

Figure (2): The design of pools

2.4.Gas chromatography analysis:

Remaining of crude oil after extraction at the end of each incubation period was measured chromatographically via tube gas chromatography using Agilent 6890 plus gas chromatograph equipped with split injector, and fused silica capillary column HP-1 of 30 m length, 0.25 µm internal diameter, and 0.5 µm film fatness. Both indicator and injector temperatures were sustained at 220°C. The column temperature was programmed to rise from 80°C to 260 °C with a rate of 5°C/min and the final time 40 min. Nitrogen/air used as a carrier gas at a flow rate of 3 mL/min.

3.RESULTS

As an outcome of total bacterial amount and viable count of triggered Bactria in broth media, the total bacterial count was 65.8 x 106 cell/ ml, whereas the viable bacterial cell was (540 cells per ml of broth media). Results of total hardness, ammonia, chloride, and phosphate are shown in Table 2.

Table (2): Results of analysis for oily wastewater before treatment by Bacteria.

Name of the parameters	Result
Total hardness	239.4 (mg $CaCO_3.l^{-1}$)
Ammonia	3.642 (ppm)
CL	636 (mg.l^{-1})
PO_4	5.05 (µgPO4-P.l^{-1})

Table (3) shows total hardness concentration for five weeks and ranged from a minimum value of 239.4 mg $CaCO_3.l^{-1}$ to a maximum amount of 256.5 mg $CaCO_3.l^{-1}$ in control containers. While in treatment pools, the value increased to 393.3 mg $CaCO_3.l^{-1}$ for 5 mg of bacteria with 10L of oily wastewater. Apparent variation was found between the first week of treatment and 5th week of treatment. Total hardness concentrations were shown in Figure (3) and Table (3).

Table (3): The results of total hardness (mg CaCO3.l^{-1}) from analyzing of oily wastewater treated by Bacteria and control.

Pool name	18 March 2019	25 March 2019	31 March 2019	7 April 2019	14 April 2019	Mean ±SD
Control	239.4	239.5	256.5	239.4	256.5	**246.26**
5 mg of bacteria with 10 L of oily wastewater	241.4	273.5	273.6	324.9	393.3	**301.34**
10 mg of bacteria with 10 L of oily wastewater	242.2	273.6	239.4	273.6	324.9	**270.74**
15 mg of bacteria with 10 L of oily wastewater	242.8	273.9	273.6	290.7	342	**284.6**

Mean ±SD	241.45	265.125	260.775	282.15	329.175	1340.8075

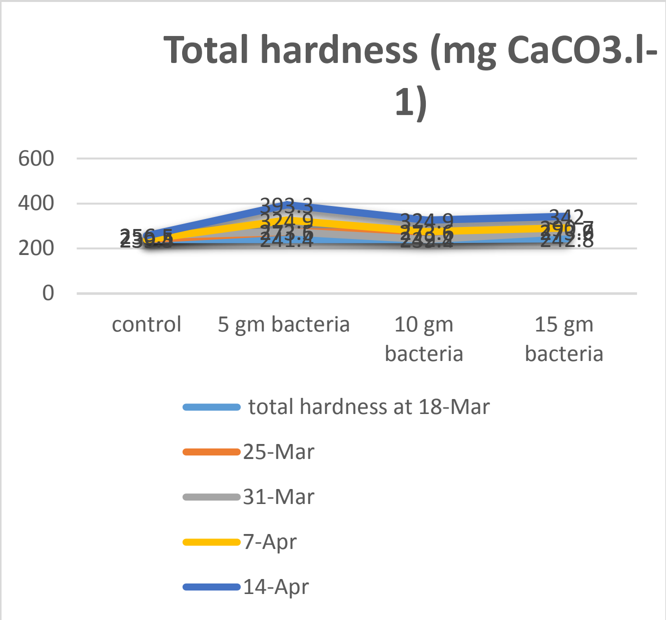

Figure (3): Total hardness in oily wastewater during the period of study

Table (4) represents the value of Ammonia in the current study; ammonia levels increased with an increase in the concentration of bacteria (Figure 4). The minimum amount of ammonia was 6.7 ppm at pools, which contains 5 mg of bacteria with 10 L of oily wastewater, while the maximum value was 10.465 ppm at pools with 15 mg of bacteria with 10 L of oily wastewater. Under the same condition with proceeding weeks, the amount of ammonia increased to a maximum value of 13.55 ppm for the pool with the highest concentration of bacteria (15 mg). This change happened only in pools that contain bacteria, while in control pools, there is no clear change. The increase in ammonia levels is continuous until the last week (5th). The maximum value of 14.811 ppm was measured at pools that contain 15 gm of bacteria, while the minimum value 13.35 ppm was measured in the first pool, which contains 5 gm of bacteria.

Table (4): The results of Ammonia (ppm) from analyzing oily wastewater treated by Bacteria consist of control.

Pool name	18 March 2019	25 March 2019	31 March 2019	7 April 2019	14 April 2019	Mean±SD
Control	6.313	6.312	6.1	4.128	5.706	**5.7118**
5 mg of bacteria with 10 L of oily wastewater	6.7	8.9	18.938	15.418	13.35	**12.6612**
10 mg of bacteria with 10 L of oily wastewater	10.023	12.868	10.683	13.597	13.42	**12.1182**
15 mg of bacteria with 10 L of oily wastewater	10.465	13.55	14.811	17.7244	14.811	**14.27228**
Mean±SD	**8.37525**	**10.4075**	**12.633**	**12.71685**	**11.82175**	**50.358915**

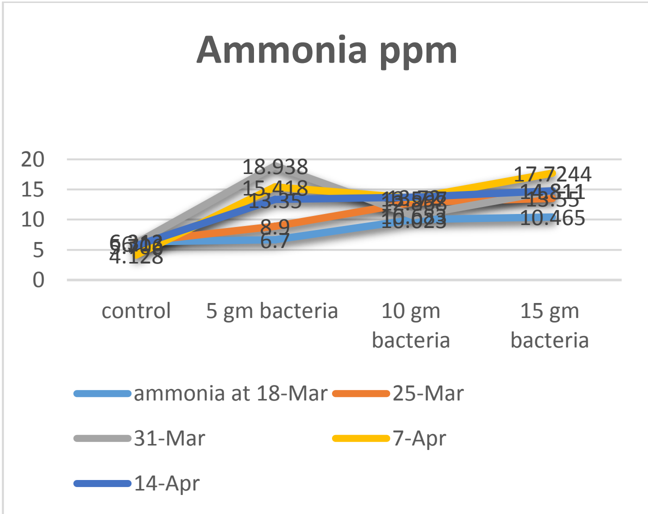

Figure (4): Ammonia levels in oily wastewater during the five weeks of bacteriological treatment.

Table (5) and Figure (5) show values of CL, which decrease along the treatment process, the same change happened to control pools. On the 14th of April, which is the last week of the procedure. The lowest value (119 mg. l^{-1}) recorded in pools that contain 5mg of bacteria, while the highest value measured in pools which contain 15 gm.

Table (5): Results of Chloride (mg. l^{-1}) in control (Row wastewater) and treated pools by Bacteria.

Pool name	18 March 2019	25 March 2019	31 March 2019	7 April 2019	14 April 2019	Mean±SD
control	636	636	148	148	140	**341.6**
5 mg of bacteria with 10 L of oily wastewater	567	564	148	120	119	**303.6**
10 mg of bacteria with 10 L of oily wastewater	490	484	108	136	136	**1354**
15 mg of bacteria with 10 L of oily wastewater	475	460	168	148	142	**278.6**
Mean±SD	**542**	**536**	**143**	**138**	**134.25**	**1885.525**

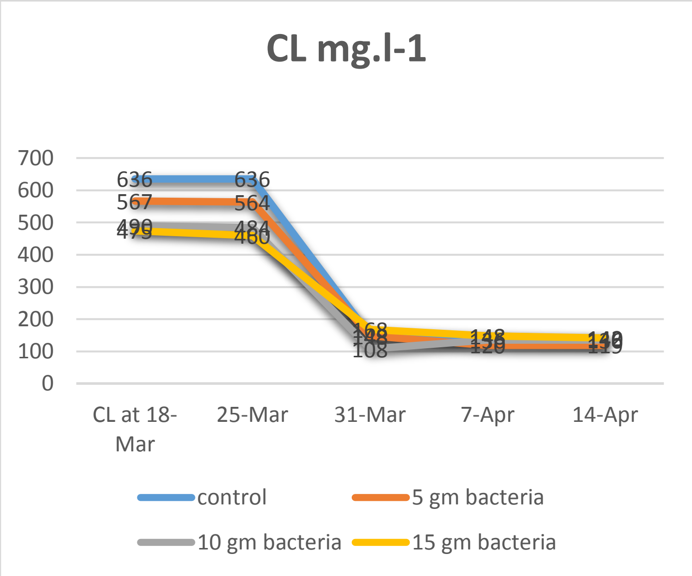

Figure (5): Chloride levels in oily wastewater during the five weeks of biological treatment.

Table (6) and Figure (6): presents PO_4 concentration, which increased during biological therapy. In the first week, the higher level of PO4 (10.5 µgPO_4-P. l^{-1}**)** presented in pools that contain 15 gm of bacteria while the lower value (7.2 µgPO_4-P. l^{-1}) was recorded in pools which contains 5 gm of bacteria. After five weeks of treatment, the amount of PO4 continuous in increasing. The highest value (54.95 µgPO_4-P. l^{-1}) measured at pools with a weight of 15 mg of bacteria. However, the control pool results decreased from 5.05 to 0.45µgPO_4-P. l^{-1}) throughout the periods of study.

Table (6): PO_4 (µgPO_4-P. l^{-1}) results for oily wastewater treated by Bacteria and control pools

Pool name	18 March 2019	25 March 2019	31 March 2019	7 April 2019	14 April 2019	Mean ±SD
control	5.05	5.04	1.7	0.8	0.45	**2.608**
5 mg of bacteria with 10 L of oily wastewater	7.2	19.9	26.2	25.3	41.55	**24.03**
10 mg of bacteria with 10 L of oily wastewater	7.8	19.75	31.4	35.15	44.35	**27.69**
15 mg of bacteria with 10 L of oily wastewater	10.5	31.55	48.9	44	54.95	**37.98**
Mean ±SD	**7.6375**	**19.06**	**27.05**	**26.3125**	**35.325**	**103.465**

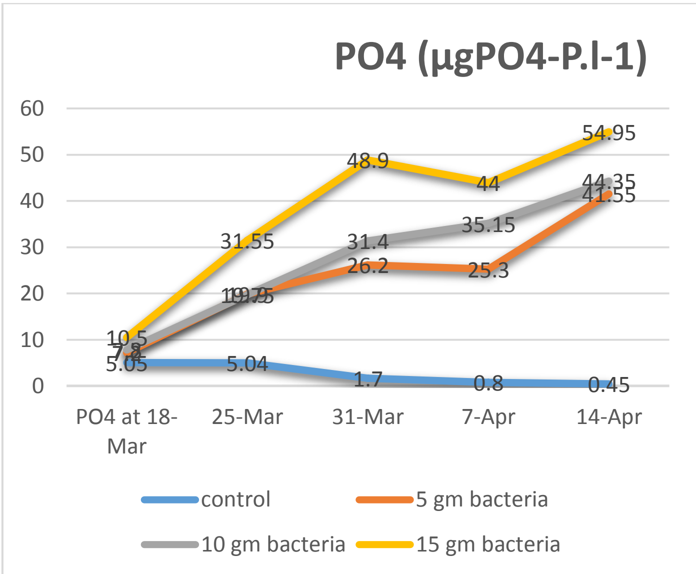

Figure (6): the amount of PO_4 in oily wastewater during the biological treatment.

Table (7): Forms and concentrations of hydrocarbon in oily wastewater of KAR oil refinery before disposal**.**

Hydrocarbon	Ret. Time	Area	Area %	µg/L
C8	4,324	42037	0.383	3830
C9	6.381	201659	1.835	18350
C10	9.009	457143	4.160	41600
C11	11.957	703764	6.405	64050
C12	14.994	554271	5.044	50440
C13	17.986	349275	3.179	31790
C14	20.869	233327	2.123	21230
C15	23.620	179851	1.637	16370
C16	26.233	168371	1.532	15320
C17	28.748	102491	0.933	9330
C18	31.685	75963	0.691	6910
C19	35.463	40537	0.369	3690
Total	64817.618	4108689	28.291	282910

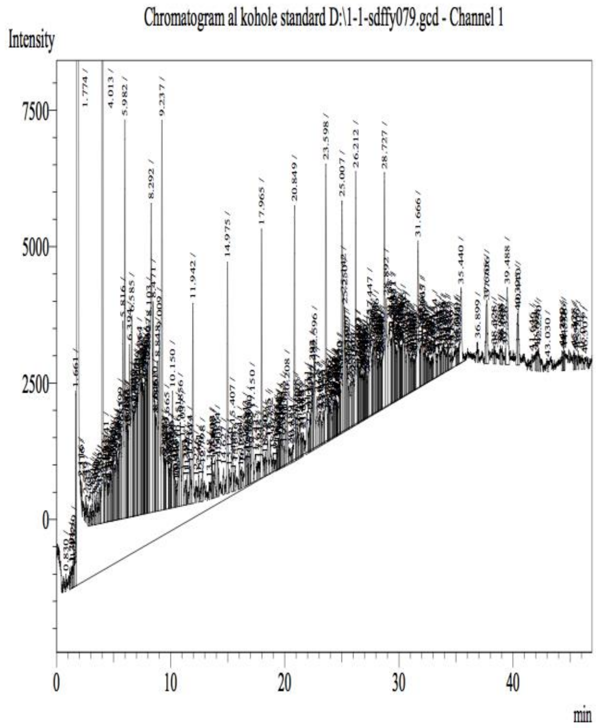

Figure (7): GC analysis graves of oily wastewater contamination without Bacteria

Figure (7) and Table (7) presents the analysis of crude wastewater with oil, and showing the oil comprised 19 hydrocarbon components plus hydrocarbon (C8, C9, C10, C11, C12, C14, C15, C16, C17, C18, and C19) which are (octane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, and nonadecane) respectively according to normal standard GC analysis of hydrocarbons within the same contain (Teng, et.al. 1994).

Table (8): The types and concentrations of hydrocarbon in oily wastewater after adding 5 gm of powder Bacteria, after two weeks of treatment.

Hydrocarbon	Ret. Time	Area of sample	Area%	µg/L
C9	6.368	31200	0.102	1020
C11	11.920	17131	0.056	560
C12	14.956	18799	0.061	610
C13	17.951	16040	0.052	520
C14	20.833	15650	0.051	510
C16	26.199	18073	0.059	590
C17	28.715	24606	0.080	800
C18	31.652	28222	0.092	920
C19	35.425	19775	0.064	640
Total	194.019	189496	0.617	6170

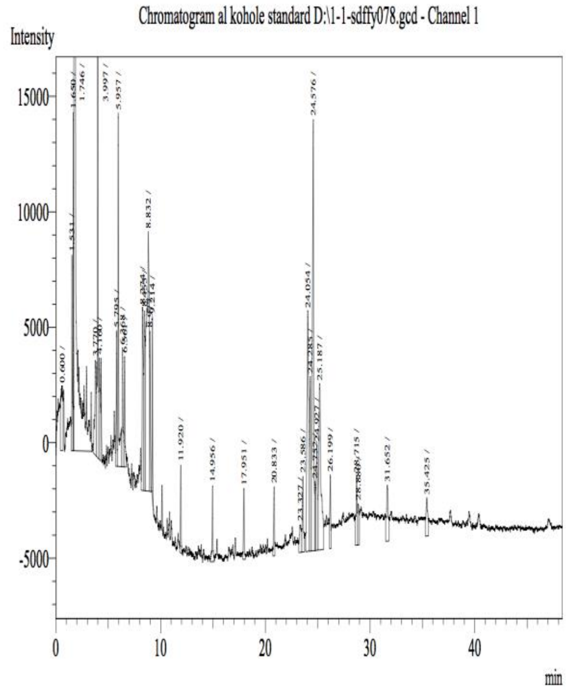

Figure (8): Sample (2) wastewater with 5gm of powder bacteria.

Figure (8): Demonstrates that the concentration of hydrocarbon after adding 5 gm of powder bacteria to 10 L of oil wastewater. Thus, the hydrocarbon declines because of the effect of bacteria to breakdown the carbon discovered in it. The hydrocarbons (C8, C10, and C15) concentration decreased to zero, while the hydrocarbon (C9, C11, C12, C13, C14, C16, C17, and C19) decrease (1020, 560, 610, 520, 510, 590, 800, 920, 640 µg/L). The remaining hydrocarbon measured by GC-MS under the same condition of standard analysis was (nonane, undecane, dodecane, tridecane, tetradecane, hexadecane, heptadecane, octadecane, nonadecane).

Table (9): The types and concentrations of hydrocarbon in oily wastewater after adding 10 gm of powder Bacteria. After two weeks of treatment.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C9	6.368	31200	0.102	1020
C11	11.920	17131	0.056	560
C12	14.956	18799	0.061	610
C13	17.951	16040	0.052	520
C14	20.833	15650	0.051	510
C16	26.199	18073	0.059	590
C17	28.715	24606	0.080	800
C18	31.652	28222	0.092	920
C19	35.425	19775	0.064	640
Total	194.019	189496	0.617	6170

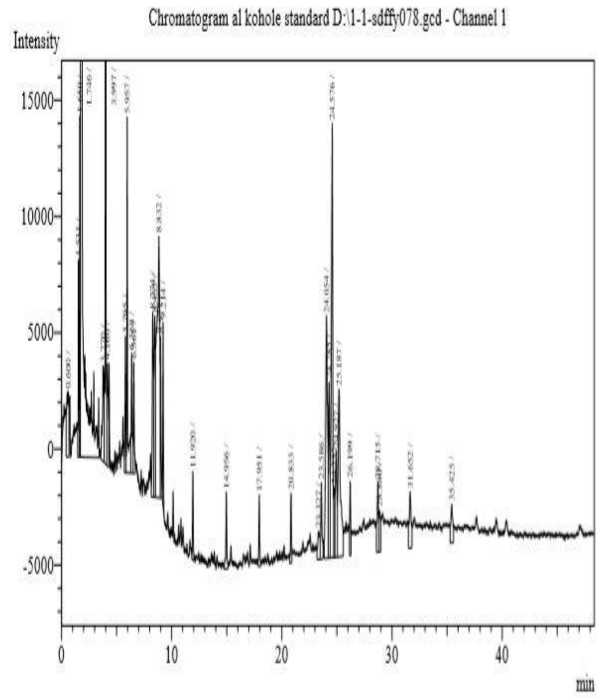

Figure (9): Sample (3) wastewater with 10 gm of powder Bacteria.

Figure 9: represents the concentration of hydrocarbon after adding 10 gm of powder bacteria to 10L of oily wastewater, the hydrocarbons (C8, C10, and C15) concentration decreased to zero, while the hydrocarbon (C9, C11, C12, C13, C14, C16, C17, and C19) decrease to (1020, 560, 610, 520, 510, 590, 800, 920, 640), which are (nonane, undecane, dodecane, tridecane, tetradecane, hexadecane, heptadecane, octadecane, nonadecane). Under the same measurement condition of standard analysis.

Table (10): The types and concentrations of hydrocarbon in oily wastewater after adding 15 gm of powder Bacteria. After two weeks of treatment**.**

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C9	6.297	14815	0.120	1200
C10	9.003	233265	1.895	18950
C11	11.933	31125	0.253	2530
C12	14.968	31736	0.258	2580
C13	17.961	23883	0.194	1940
C14	20.842	22548	0.183	1830
C15	23.593	23194	0.188	1880
C16	26.208	17813	0.145	1450
C17	28.720	14637	0.119	1190
C18	31.668	13767	0.112	1120
C19	35.431	13352	0.108	1080
Total	226.624	1379255868	3.575	35750

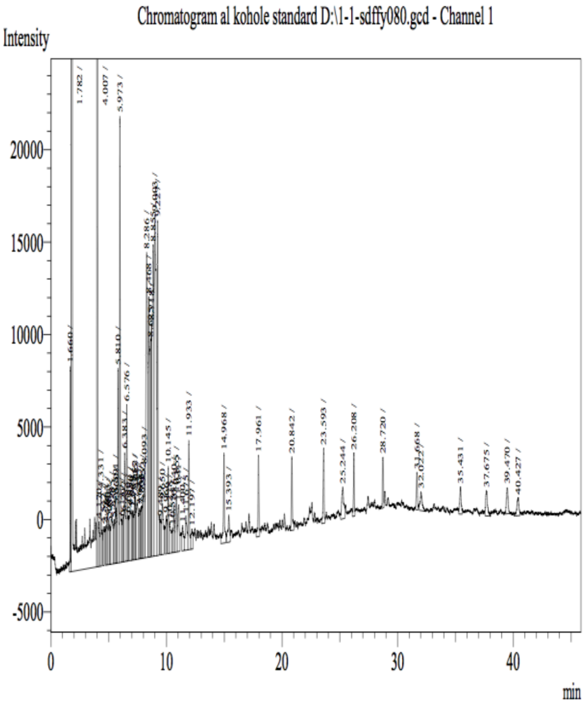

Figure (10): Sample (4) wastewater with 15gm of powder bacteria)

Figure 10: Shows the amount of hydrocarbon after adding 15 ml of broth bacteria to 200 ml of oily wastewater, the hydrocarbons (C8 and C9) concentration decreased to zero, while the hydrocarbon (C10, C11, C12, C13, C14, C15, C16, C17, C18, and C19) became (1200,18950, 2530, 2580, 1940, 1830, 1880, 1450, 1190, 1120, 1080 µg/L) which are (nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, and nonadecane). Under the same measurement condition of standard analysis.

Table (11): Types and concentrations of hydrocarbon in oily wastewater for control containor, which is without bacteria.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C10	8.999	58546	0.272	2720
C11	11.934	66613	0.310	3100
C12	14.968	69515	0.323	3230
C13	17.958	57673	0.268	2680
C14	20.844	52667	0.245	2450
C16	26.211	50160	0.233	2330
C17	28.728	49710	0.231	2310
C18	31.665	38595	0.180	1800
C19	35.433	24137	0.112	1120
Total	196.74	467616	2.174	21740

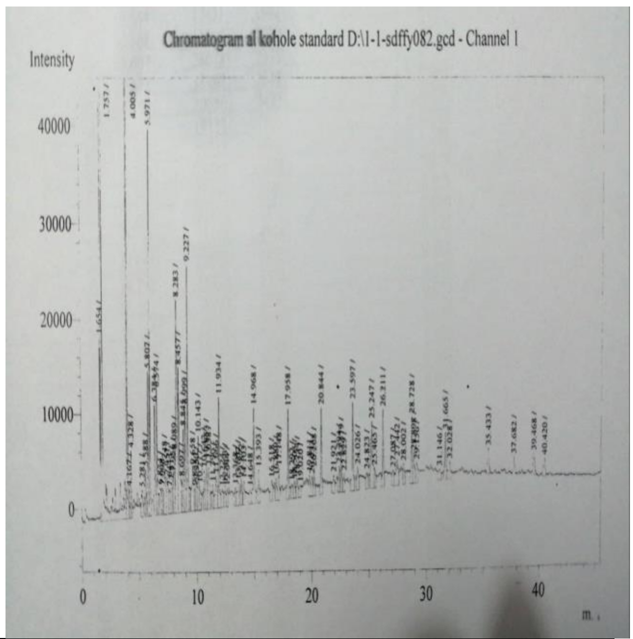

Figure (11): Sample (5) is the control of oily wastewater without Bacteria only under aeration condition.

Figure (11): this figure represents control. Which is the oily wastewater without Bacteria only under aeration and the same temperature condition. After two weeks of leaving this wastewater, the result of GC-MS shows that the hydrocarbon (C8, C9, and C15) disappear just by aeration process. Still, other hydrocarbon value (C10, C11, C12, C13, C14, C16, C17, C18, and C19) became (2720, 3100, 3230, 2680, 2450, 2330, 2310, 1800, 1120), which are (nonane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, heptadecane, octadecane, and nonadecane).

Table (12): The types and concentrations of hydrocarbon in oily wastewater after adding 5 gm bacteria after three weeks of treatment.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C9	5.996	28613	0.030	300
C13	17.479	1310	0.001	10
C17	28.545	24406	0.025	250
C18	31.570	8240	0.009	90
C19	35.242	22409	0.023	230
Total	118.832	84978	0.0799	880

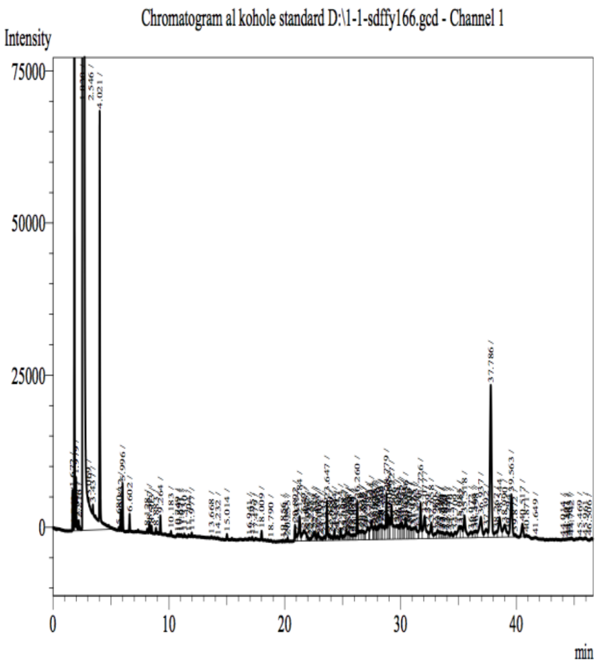

Figure (12): Sample (1) oily wastewater with 5 gm of Bacteria after 3 weeks of treating, under aeration condition.

Table (12) and Figure (12) shows the remaining hydrocarbons after three weeks of treating oily wastewater by 5 gm of bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C10, C11, C12, C14, C15 andC16) disappear, while the remaining hydrocarbons (C9, C13, C17, C18 and C19) became (300, 10, 250, 90, 230) which are (nonane, heptadecane, octadecane, nonadecane).

Table (13): The types and concentrations of hydrocarbon in oily wastewater with 10 gm of bacteria after three weeks.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C11	11.702	4519	0.006	60
C13	17.473	4909	0.007	70
C17	28.517	15669	0.022	220
C18	31.561	1389	0.002	20
C19	35.225	13170	0.019	190
Total	124.478	39656	0.056	560

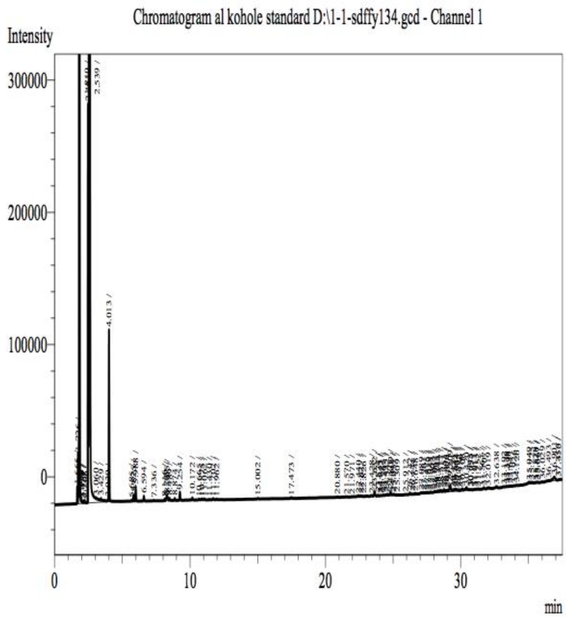

Figure (13): Sample (2) oily wastewater with 10 gm of Bacteria, under aeration condition.

Table (13) and figure (13) shows the remaining hydrocarbons after three weeks of treating oily wastewater by 10 gm of bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C9, C10, C12, C14, C15, and C16) disappear, while the remaining hydrocarbons (C11, C13, C17, C18 and C19) became (60,70,220,20,190) which are (undecane, tridecane, heptadecane octadecane nonadecane).

Table (14): The types and concentrations of hydrocarbon in oily wastewater after three weeks of treating with 15 gm of bacteria under aeration condition.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C10	8.894	1648	0.002	20
C17	28.528	2382	0.002	20
C18	31.213	13650	0.013	130
C19	35.092	20301	0.020	200
Total	103.727	37981	0.037	370

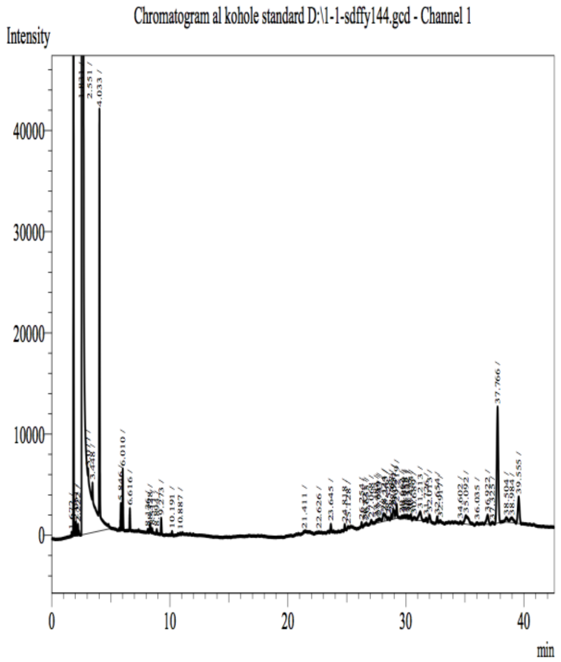

Figure (14): Sample (3) oily wastewater contains 15 gm of Bacteria after three weeks of treatment, under aeration condition.

Table (14) and Figure (14) shows the remaining hydrocarbons after three weeks of treating oily wastewater by 15 gm of bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C9, C11, C12, C13, C14, C15, and C16) disappear, while the remaining hydrocarbons (C10, C17, C18 and C19) became (20, 20, 130, 200) which are (decane, heptadecane, octadecane, and nonadecane).

Table (15): The types and concentrations of hydrocarbon in oily wastewater (control) without bacteria, after three weeks of treating.

Hydrocarbon	Ret. Time	Area	Area %	µg/L
C11	11.715	1303	0.002	20
C13	17.485	1304	0.002	20
C16	26.035	4667	0.007	70
C17	28.533	8355	0.013	130
C18	31.241	2068	0.003	30
C19	35.215	5468	0.008	80
Total	150.224	23165	0.035	350

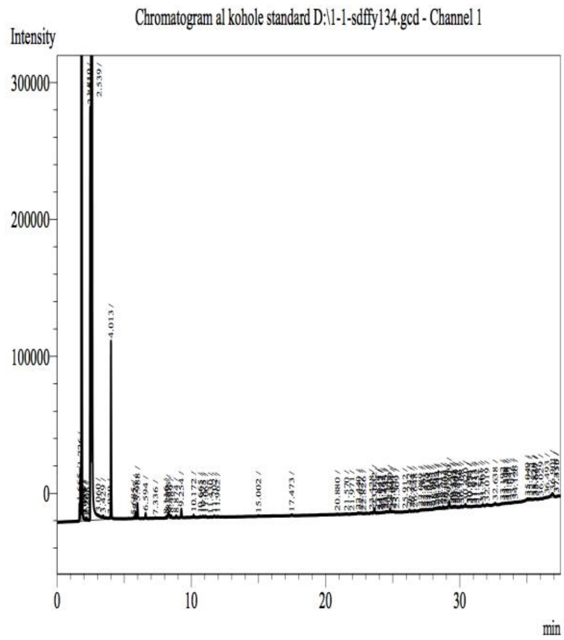

Figure (15): Sample (4) control of oily wastewater without Bacteria only under aeration condition.

Table (15) and Figure (15) shows the remaining hydrocarbons after three weeks of treating oily wastewater without bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C9, C10, C12, C14, C15) disappear, while the remaining hydrocarbons (C11, C13, C16, C17, C18, C19) became (20,20,70,130,30,80) which are (undecane, tridecane, hexadecane, heptadecane octadecane nonadecane).

Table (16): The types and concentrations of hydrocarbon in oily wastewater with 5gm of bacteria after four weeks of treatment.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C9	6.231	8385	0.011	110
C13	17.861	1114	0.001	10
C17	28.616	16539	0.021	210
C18	31.000	1649	0.002	20
C19	35.067	3495	0.004	40
Total	118.775	31182	0.039	390

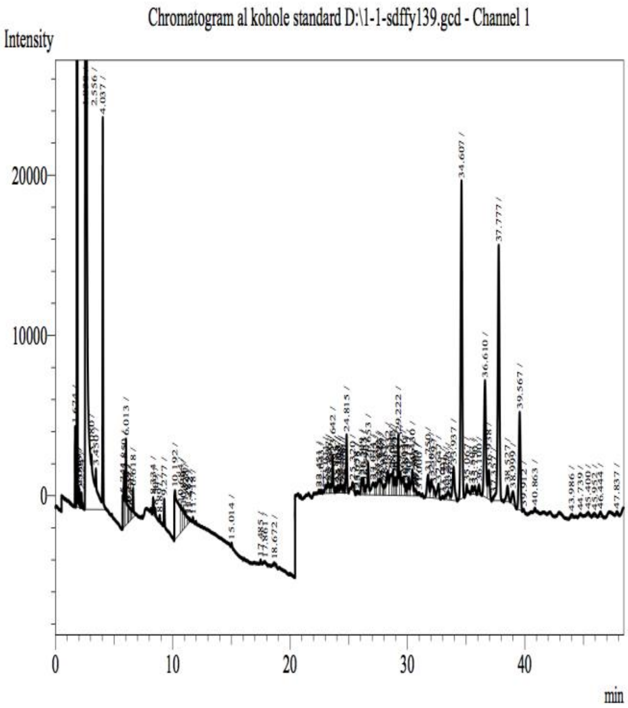

Figure (16): Sample (1) oily wastewater contains 5gm of Bacteria, after four weeks under aeration condition**.**

Table (16) and Figure (16) shows the remaining hydrocarbons after four weeks of treating oily wastewater with 5 gm bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C10, C12, C14, C15, C16) disappear, while the remaining hydrocarbons (C9, C13, C17, C18, C19) became (110, 10, 210, 20, 40) which are (nonane, tridecane, heptadecane octadecane nonadecane).

Table (17): the types and concentrations of hydrocarbon in oily wastewater with 10 gm of bacteria, after four weeks from treatment.

Hydrocarbon	Ret. Time	Area	Area %	µg/L
C11	11.720	4957	0.005	50
C17	28.531	6980	0.007	70
C18	31.262	1544	0.001	10
C19	35.231	16503	0.016	160
Total	106.744	29984	0.029	290

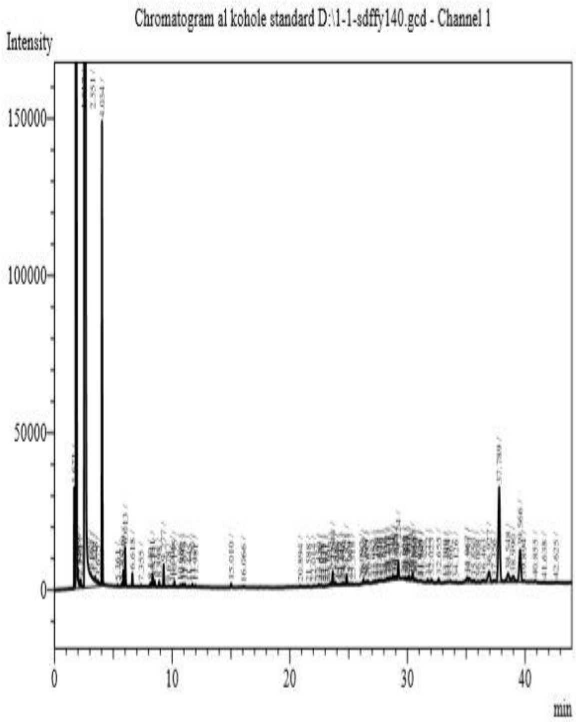

Figure (17): Sample (2) control of oily wastewater with 10 gm of Bacteria after 4 weeks of treatment.

Table (17) and Figure (17) shows the remaining hydrocarbons after four weeks of treating oily wastewater with 10 gm bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C9, C10, C12, C13, C14, C15, C16) disappear, while the remaining hydrocarbons (C11, C17, C18, C19) became (50, 70, 10, 160) which are (undecane, heptadecane, octadecane, nonadecane).

Table (18): the types and concentrations of hydrocarbon in oily wastewater with 15 gm of bacteria, after four weeks of treatment.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C10	8.511	1042	0.001	10
C17	28.514	4058	0,004	40
C18	31.060	16447	0.014	140
C19	35.241	4463	0.004	40
Total	103.326	26010	0.023	230

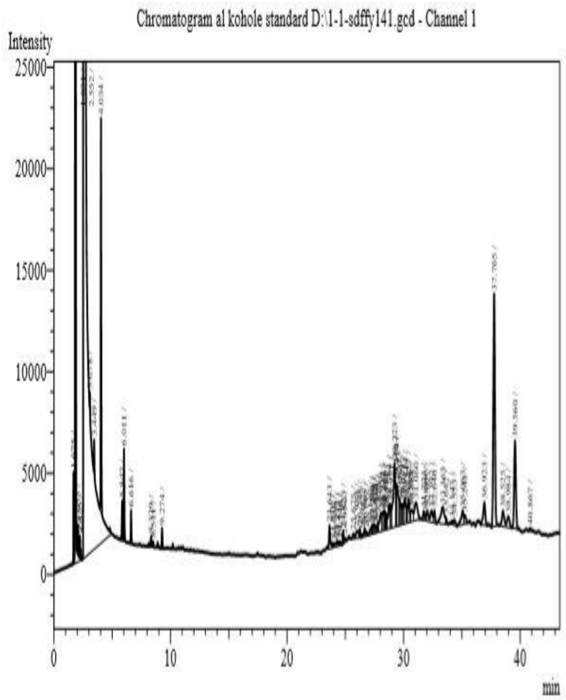

Figure (18): Sample (3) oily wastewater contains15 gm of Bacteria after four weeks of treatment, under aeration condition.

Table (18) and Figure (18) shows the remaining hydrocarbons after four weeks of treating oily wastewater with 15 gm bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C9, C11, C12, C13, C14, C15, C16) disappear, while the remaining hydrocarbons (C10, C17, C18, C19) became (10, 40, 140, 40) which are (decane, heptadecane, octadecane, nonadecane).

Table (19): The types and concentrations of hydrocarbon in oily wastewater. In addition to the control, which is without bacteria.after four weeks.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C11	11.719	1649	0.002	20
C13	17.493	2310	0.002	20
C17	28.537	6809	0.007	70
C19	35.229	3629	0.004	40
Total	92.978	14397	0.015	150

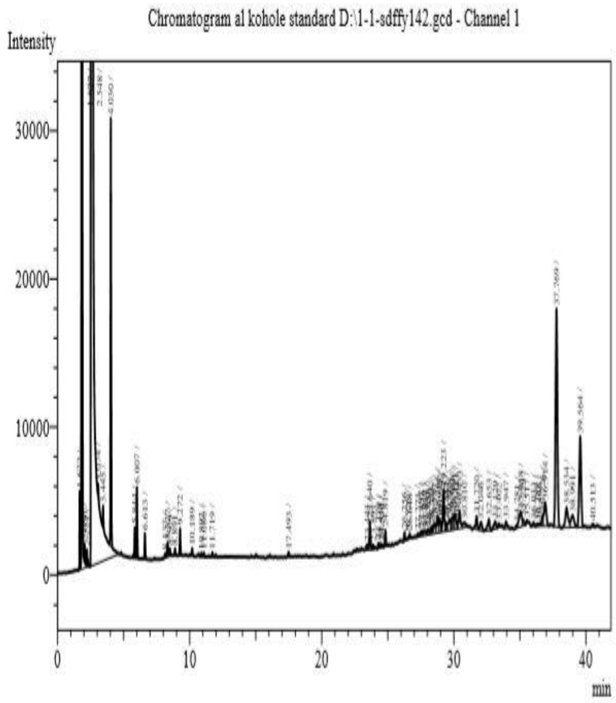

Figure (19): Sample (4) control of oily wastewater without Bacteria only under aeration condition. After four weeks of treatment.

Table (19) and Figure (19) shows the remaining hydrocarbons after four weeks of treating oily wastewater without bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C9, C10, C12, C14, C15, C16) disappear, while the remaining hydrocarbons (C11, C13, C17, C19) became (20, 20, 70, 40) which are (undecane, tridecane, heptadecane, octadecane, nonadecane).

Table (20): The types and concentrations of hydrocarbon in oily wastewater with 5 gm of bacteria after five weeks.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C9	6.008	41179	0.119	1190
C17	28.522	6360	0.018	180
C19	35.245	14049	0.041	410
Total	69.775	61588	0.178	1780

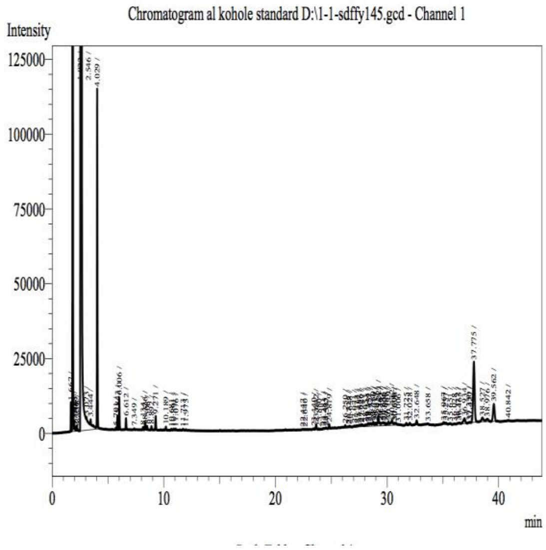

Figure (20): Sample (1) control of oily wastewater with 5 gm of Bacteria under aeration condition. After five weeks of treatment.

Table (20) and Figure (20) shows the remaining hydrocarbons after five weeks of treating oily wastewater with 5 gm bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C10, C11, C12, C13, C14, C15, C16, C18) disappear, while the remaining hydrocarbons (C9, C17, C19) became (1190,180,410) which are (nonane, heptadecane, nonadecane).

Table (21): the types and concentrations of hydrocarbon in oily wastewater contain 10 gm of bacteria under aeration condition after five weeks of treatment.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C11	11.715	2742	0.002	20
C17	28.519	11816	0.010	100
C19	35.240	12588	0.011	110
Total	75.474	27146	0.023	230

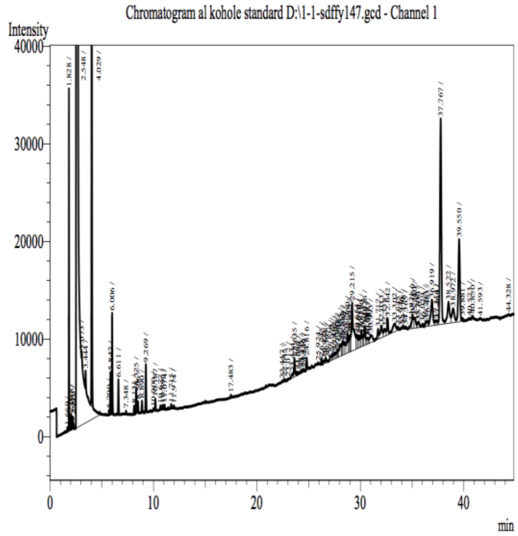

Figure (21): Sample (2) control of oily wastewater with 10 gm of Bacteria after five weeks of treatment.

Table (21) and Figure (21) shows the remaining hydrocarbons after five weeks of treating oily wastewater with 10 gm bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C9, C10, C12, C13, C14, C15, C16, C18) disappear, while the remaining hydrocarbons (C11, C17, C19) became (20, 100, 110) which are (undecane, heptadecane, nonadecane).

Table (22): the types and concentrations of hydrocarbon in oily wastewater contain 15 gm of bacteria with aeration. After five weeks of treatment.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C19	35.076	1697	0.027	270

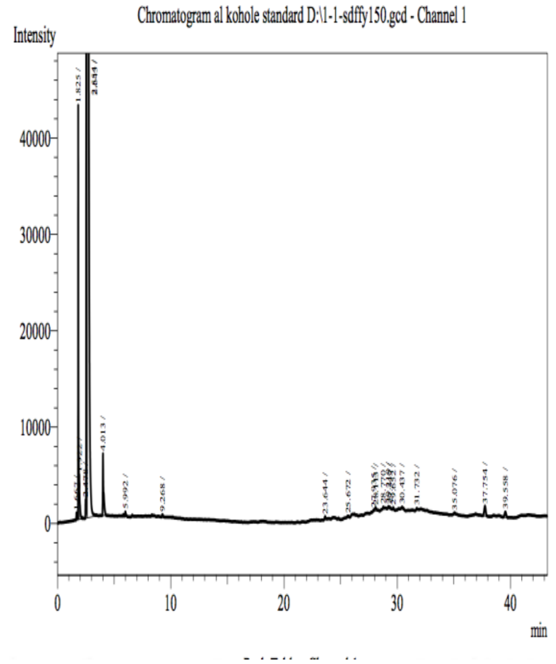

Figure (22): Sample (3) control of oily wastewater with 15 gm of Bacteria under aeration condition.

Table (22) and Figure (22) shows the remaining hydrocarbons after five weeks of treating oily wastewater with 10 gm bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C9, C10, C11, C12, C13, C14, C15, C16, C18) disappear, while the remaining hydrocarbons (C19) became (270) which are (nonadecane).

Table (23): The types and concentrations of hydrocarbon in oily wastewater (control) without bacteria. After five weeks of treatment.

Hydrocarbon	Ret. Time	Area	Area%	µg/L
C11	17.483	1030	0.001	10
C18	31.552	1810	0.002	20
C19	35.224	5147	0.005	50
Total	84.259	7987	0.008	80

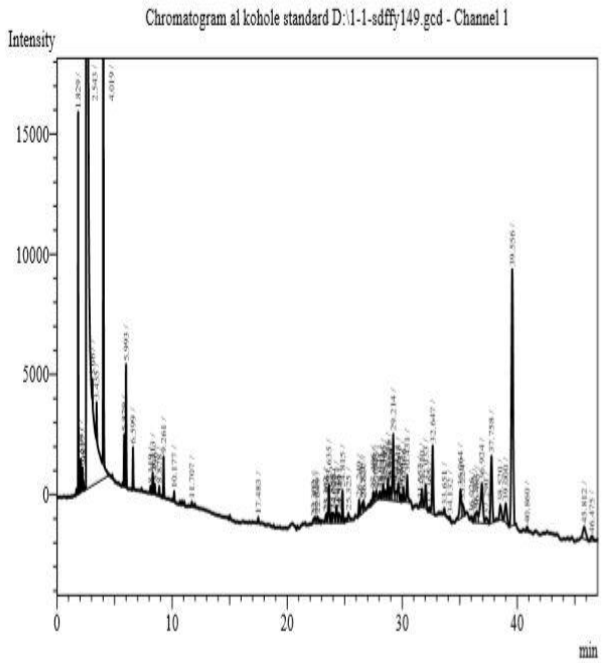

Figure (23): Sample (4) control of oily wastewater without Bacteria only under aeration condition. After five weeks of treatment.

Table (23) and Figure (23) shows the remaining hydrocarbons after five weeks of treating oily wastewater with 10 gm bacteria under aeration and the same temperature condition. The results of GC-MS represent that hydrocarbon (C8, C9, C10, C12, C13, C14, C15, C16, C17) disappear, while the remaining hydrocarbons (C11, C18, C19) became (10,20,50) which are (undecane, octadecane, nonadecane).

Discussion:

The GC- MS outcomes indicates that the hydrocarbon (C8, C9, C10, C11, C12, C14, C15, C16, C17, C18, and C19) in oil wastewater before bioremediation treatment with different highest peaks meaning types of hydrocarbons found as a result of a method of crude purification oil in the refiner. However, these hydrocarbons uncovered in complex wastewater products (Akpor *et.al.,* 2014)

Microorganisms are crucial to the degradation of petroleum hydrocarbons, and that they largely affect the transformation and fate of petroleum hydrocarbons in the environment. While some broad bacteria spectrum of petroleum hydrocarbon degradation ability (Xu. *et al.*, 2018). The degradation of petroleum hydrocarbons can be mediated by a specific enzyme system (Fritsche and Hofrichter, 2000). Other mechanisms involved are an attachment of microbial cells to the substrate and the production of biosurfactants. The uptake mechanism linked to the attachment of the cell to the oil droplet is still unknown, but the production of biosurfactant has been well studied (Das and Chandran, 2011). The enzyme involved in biodegradation of petroleum hydrocarbons have specialty such as soluble methane monooxygenases degrade C1-C8 (McDonald, *et al.*, 2006), alkB related Alkane hydroxylases substrate is C5-C16 (Jan, *et al.*, 2003), Dioxygenases substrate C10-C30 Alkanes (Jan, *et al.*, 1996). The difference in the results of upper carves as shown C8 and C9 remain mainly after bioremediation by bacteria due to the enzymes, which produce, by bacteria and their activity.

Although from the activity of bacteria to remove hydrocarbon, there is another chemical change that happened to the treated water.

According to Table (3) and Figure (3), the number of total hardness increases because of the rise of suspended particles of degraded drops of oil. As mentioned above, the enzyme produced by bacteria makes the coagulated drops of oil degraded and became particles easy to consume by bacteria. So this particle makes the reading of total hardness increase (Fakhru’l-Razi, et al., 2009).

The amount of ammonia changed with the time of treatment, as shown in Figure (4). The amount of ammonia in the first weeks increased; after that, with duration, we can see at the last week the ammonia decreased in all the pools of oily wastewater, which have different weights of bacteria. These changes are due to oxidizing ammonia by Bacillus bacteria as a source of energy for bacteria growth. This result then became the reason for choosing Bacillus to be used in the wastewater treatment system. Because it will not create pollution if the oily wastewater produced by this treatment discharging to the river, furthermore, Bacillus can be selected for industrial wastewater treatment system on a broad scale (Wardhani, 2017).

According to the chemical analysis, the amount of PO4 increased with increasing the amount of bacteria to the wastewater. This increase happened because some microorganisms like *Bacillus subtitles* bacteria release little P in their natural state. However, these microorganisms can increase the concentration of available P by secreting organic acids and various degrading enzymes (Phytase, nuclease, phosphatase, etc.) to decompose insoluble phosphate in the oily wastewater (Wu *et al.*, 2019). Also, this is like an indicator that shows that the bacteria are active, and there is an obvious effect in treatment.

Chloride for safety reasons, chloride in wastewater should not exceed 350 mg/L as directed and WHO Standard (WHO, 2006). In water bodies, elevated chloride levels can threaten the sustainability of ecological food sources, hence posing a risk to species survival, growth as well as reproduction. Bioaccumulation and persistence of chloride may affect aquatic organisms and water quality (Imo, 2017). The biological treatment by bacillus bacteria reduced the amount of chloride compared to the control because the CL is essential for the growth of bacteria (Roeßler, 2003).

The result of our study showed that 15 mg of Bacteria growth or bacterial number displaying more capabilities for the bioremediation of petroleum oil-contaminated water. In recent times, rapidly and achieved significant gains, microbial remediation technology has developed.

Conclusion:

To sum-up, petroleum hydrocarbons resulting in wastewater can be seen as one of the most dangerous pollutants due to their high toxicity and their effects on human comfort and environmental health. Bioremediation by petroleum hydrocarbon-degrading bacteria is generally regarded as an eco-friendly and efficient technology.

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