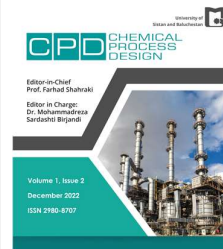




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Degradation of Oily Sludge using Bioremediation in Slurry Phase

Zeynab Honarvar, Mohammad Reza Miroliaei*, Amir Sarrafi, Seyed Ahmad Ataei

Department of Chemical Engineering, Faculty of Engineering, Shahid Bahonar University of Kerman, Kerman, Iran

ARTICLE INFO

Article history:

Received: 2022-10-13

Received in revised form: 2023-01-13

Accepted: 2023-01-26

Published online: 2023-01-27

Keywords:

Bioremediation; Slurry phase; Oily sludge; Hydrocarbon; Wastewater; Tween 80.

DOI: 10.22111/CPD.2023.43687.1011

ABSTRACT

Oily sludge is one of the major oil pollutants that bioremediation is considered the most efficient way of treating and removing harmful hydrocarbons from these pollutants. In this study, the biodegradation of oil sludge from the Sirjan Gasoil storage reservoir in the slurry phase was investigated. In this research, the effect of sewage (wastewater) and surfactant and their interactions on biodegradation in the slurry phase has been investigated. The oil sludge was mixed with a 25% (w/v) combined ratio with different liquids and the slurry phase was applied for 21 days by incubator shaker at 130, 150, and 190 rpm. The temperature of the specimens was set in the appropriate range for the activity of degrading bacteria (30°C). Determination of the amount of hydrocarbons by dichloromethane extraction was carried out every week and an incremental removal of hydrocarbon was observed during this period. Among the samples, the highest removal of hydrocarbons was attributed to a rotational speed of 190 rpm with a removal rate of approximately 55%.

1. Introduction

Crude oil as a source of energy is a necessary part of the modern world. It will be the main source of energy for decades to come, so there is a need to control the amount and type of leakage caused by the use of oil and its products [1]. Petroleum hydrocarbons are mainly made of hydrogen and carbon, with a ratio of 2 hydrogen atoms to 1 carbon atom, hence they are called hydrocarbons [2]. These complex compounds are basically a combination of various hydrocarbons. After extracting oil due to the change in temperature, and the separation of the associated gas, the thermodynamic equilibrium of the crude oil components is shattered and the heavier compounds begin to precipitate [3]. The oily sludge is a complex combination of hydrocarbons, and solids, including heavy metal compounds. Due to the presence of these compounds, oily sludge is carcinogenic and considered a potent toxicant for human

immunological systems. Therefore, today, due to environmental impacts and human health risks, contaminated places with oily sludge have been taken into consideration [4]. In petroleum refineries, oily sludge is produced in fluidized bed catalytic cracking [5]. The amount of sludge produced from oil refining processes depends on various factors such as crude properties (density and viscosity), refinery process design, and refinery capacity [6]. The formation of oily sludge in crude oil storage tanks results in the capture of a high percentage of this precious material in tankers. Increasing the accumulation of sediment can increase the rate of corrosion at the bottom of the reservoirs. Normal alkanes, branched alkanes, and annular alkanes enter irreversible damage to the respiratory, renal, and nervous systems. Low molecular-weight aromatic hydrocarbons are usually associated with acute toxicity, while aromatic hydrocarbons with high molecular weight have been reported mutagenesis and lethal [7].

In general, physical, chemical, and biological technologies are used for oily sludge treatment, which can be mentioned in incineration, stabilization and solidification, oxidation treatment, phytoremediation, and bioremediation. With the advancement of biological methods, more restrictions were found for the use of physical and chemical methods. Compared to these methods, biological methods are environmentally friendly and affordable. Although each method has its own advantages and disadvantages, the cost of complex reactions and equipment during the use of physical and chemical methods, the complexity of processes, and the increase in operational costs are inevitable. Biodegradation of oily sludge usually saves energy, investment, and operating costs. More than 50% of the Superfund sites have chosen biological technology as a suitable treatment option [8-10]. The rate of biodegradability of hydrocarbons is influenced by several parameters. Therefore, it is necessary to understand the limiting or increasing factors of the microbial population in order to optimize the bioremediation process [11]. The efficiency of bioremediation of hydrocarbons by microorganisms depends on factors such as the structure and nature of pollutant hydrocarbons, environmental conditions, and the characteristics of existing microbial collections. In simple biological degradation systems that require little microbiological expertise, the limiting factors are mainly the amount of available nutrients and oxygen or the lack of uniformity of the contaminated environment [12].

According to recent studies, operational methods of bioremediation include land farming, composting/bio piling, and bio slurry. Land farming is a simple method in which contaminated soils are spread over prepared bedding, and periodically they move to disperse pollutants [13]. Bio piling leads to the transformation of waste into clusters or clumps, usually at a height of two or four meters, for decomposition using indigenous or extrinsic microorganisms. In composting, the contaminated soil is mixed with non-hazardous organic additives such as manure, agricultural waste or food (rich in carbon), organic nitrogen, mineral compounds, and phosphorus required for the growth of microorganisms [14]. The efficiency of microorganisms in the degradation of hydrocarbons in the biological recovery of soil and solid phase is rarely investigated through biodegradation of the slurry phase. The water phase in the slurry reactor is increased to increase the physical mixing, while in the solid phase, only the nutrients are added and the soil is mechanically aerated at different distances. Bioremediation in the liquid phase is much faster than other bioremediation techniques and is very useful in cases where contamination must be rapidly decomposed [6].

Slurry reactor technology mixes sludge and water in a reactor to achieve greater dissolution and, consequently, more degradation of contamination. The use of this method increases the bioavailability of pollutants, electron receptors, and nutrients for the microbial population and provides the most suitable conditions for the treatment of pollutants. Reducing the concentration of pollutants in the slurry reactor in comparison with solid phase reactors is considerably

higher, which is due to increased mass transfer between liquid and solid phases [15]. This study was aimed at purifying sludge and reducing and eliminating existing pollutants in order to prevent the accumulation of oil pollutants and help protect the environment. This research, it was tried to provide a possibility to increase the rate of decomposition of hydrocarbons in oily sludge by creating a slurry phase and biodegradation at different speeds of motion. It is also expected to increase removal efficiency by using sewage in the slurry phase.

2. Materials and Methods

2.1. Materials

The oily sludge was dredged from the gas oil and kerosene reservoirs of the National Iranian Oil Products Distribution Company (NIOPDC) of the Sirjan region of Kerman, Iran. It was a complicated mix of hydrocarbons, water, and sediments. Water and petroleum liquid in the sludge was separated based on differences in density. Then, the residue of the sludge (sediment) was placed in ambient temperature conditions for 3 months until volatiles were removed completely by evaporation. According to the ASTM D1796 water and sediment test, the petroleum sludge specifications were 10% petroleum liquid, 65% water, and 25% sediment. The solvent used in these tests of liquid-solid extraction was dichloromethane, which was purchased from Sigma Aldrich (NSW, Australia). Nitric acid was purchased from Merck Germany. Wastewater was collected from the septic of Kerman university; its characteristics was listed in Table 1.

Table 1. Analytical of wastewater was used in this research (mg/L)

Factor	COD	BOD	TSS	EC	NO ₂	NO ₃	pH	PO ₄	SO ₄	AS	Cd	Ni	Zn
Value	191	92	34	1932	0.03	0.018	7.96	17.56	7.335	2	0.034	0.095	0.0185

2.2. Analytical methods

For the determination of physicochemical characteristics of oily sludge, the moisture content was determined by the ASTM D4959-16 standard, the pH by ASTM D4980-89, the organic carbon using the Loss-On-Ignition (LOI) [16], nitrogen by Kjeldahl method [17], phosphorus by colorimetric method, and using a spectrophotometer [16] and heavy metal, copper, lead, chromium, nickel and zinc were measured using atomic absorption spectroscopy [18].

2.3. Apparatus

Hydrocarbon measurements were performed using a gas chromatograph (GC), Varian CP-3800 connected to a flame ionization detector (FID). The elemental combustion system was the Costech model ECS4010 with an auto-sampler (Bremen, Germany). A laboratory mixer and shaker model KS 4000i control (IKA, Germany) provided a speed range from 10 to 500rpm [19].

2.4. Sample preparation

A beaker (400mL) was used as test unit. 40g of the dried sludge was taken and added to 160 mL of liquid phase into the test unit. Samples were made based on 25% (w/v), as listed in Table 2. After adjusting the temperature, the samples were tested at three different speeds 130, 150, and 190 rpm for 21 days into a shaker incubator. 0.3mL of Tween 80 surfactant was added to some samples for reduce the surface tension between the two phases and improve the biodegradation.

2.5. Total petroleum hydrocarbon (TPH) measurements

2g of the dried sample added to Erlenmeyer flask. Then, the sample was placed in a shaker for 30min at 150rpm. After 30min, 10mL dichloromethane was added to the sample and again shaken for 30min, this process was repeated 3 times. Afterward, the sample was centrifuged, and the extract was poured into a weighed flask. The residue was weighed after drying in an oven (to evaporate the solvent) at 45°C for a day. The total amount and removal percentage of petroleum hydrocarbons were calculated according to equations 1 and 2 [20].

$$\text{TPH(mg/kg dry sludge)} = \frac{(\text{Empty test tube} - \text{Test tube with hydrocarbon})(\text{mg}) \times 1000}{\text{mass of dry sludge (g)}} \quad (1)$$

$$\text{Removal percentage} = \frac{\text{The initial hydrocarbon} - \text{The final hydrocarbon}}{\text{The initial hydrocarbon}} \times 100 \quad (2)$$

Table 2. Experimental factors

Samples	Liquid phase			
	Distillated water (mL)	Wastewater (mL)	Oily liquid (mL)	Tween 80 (mL)
1	160	0	0	0
2	159.7	0	0	0.3
3	0	160	0	0
4	0	0	160	0
5	0	159.7	0	0.3
6	0	0	159.7	0.3
7	0	80	80	0
8	0	79.85	79.85	0.3

3. Results and discussion

3.1. Characterization of oily sludge

Physicochemical characteristics of the oily sludge are shown in Tables 3 and 4.

Table 3. Characterization of oily sludge

Parameter	Content (%)
pH	7.02
Initial moisture	20.3
Organic matter	54.99
Carbon (C)	31.902
Nitrogen (N)	0.17
Phosphorus (P)	0.05
Total hydrocarbon (TPH)	25.7

Table 4. Heavy metal concentrations

Heavy metal	Oily sludge (ppm)	Max. permitted concentration (ppm)
Cu	0.137	3.5
Pb	0.44	55
Ni	0.2	2.6
Zn	2	16
Cr	0.806	3.8

The pH of the sludge is within the range for biological activity of microorganisms (6.5-8.5). Consequently, there is no need adding buffer adjusting the pH of the environment. In the studied sludge, the ratio of carbon to nitrogen and phosphorus, which is the important food source for the activity of microorganisms, (100:10:1) is considered [21]. The ratio of carbon to nitrogen (C/N) is approximately 187.66, which is much higher than the appropriate level, indicating the low nitrogen content of the oily sludge. The amount of phosphorus in the oily sludge is low compared to carbon and nitrogen, and it means the limitation of the amount of phosphorus and nitrogen in the process, as a result increase

in the amount of removal by adding nutrient sources is desirable. The total amount of hydrocarbons in the sludge was 257,000 (mg/kg of dry sludge), which has been attempted to reduce this amount by performing a bioremediation process. The concentration of heavy metals in the primary sludge was measured and was within the permitted range of environmental protection organizations [22]; therefore, no experiment was conducted to reduce the concentration of heavy metals. In order to identify the compounds in petroleum sludge, a gas chromatography/mass spectrometry was used. Fig. 1 and Table 5 are shown a complex of hydrocarbon compounds of primary sludge, that extracted by dichloromethane solvent.

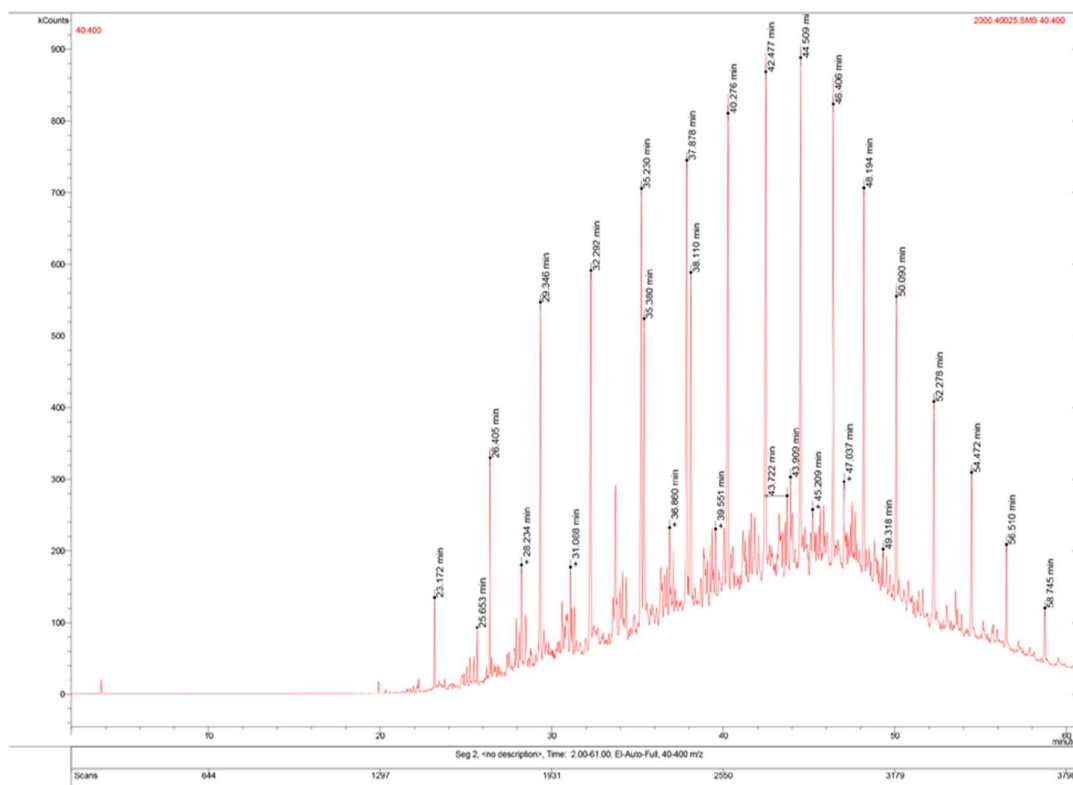


Fig. 1. Gas chromatography with mass spectrometry of primary sludge

Table 5. Possible compounds of primary oil sludge

Third guess	Second guess	First guess	Retention time (min)
Nonadecane (C ₁₉ H ₄₀)	1-Decanol, 2-hexyl (C ₁₆ H ₃₄ O)	2-Hexyl-1-octanol (C ₁₄ H ₃₀ O)	35.237
1-Decanol, 2-hexyl (C ₁₆ H ₃₄ O)	2-Hexyl-1-octanol (C ₁₄ H ₃₀ O)	1-Decanol, 2-hexyl (C ₁₆ H ₃₄ O)	37.886
Eicosane, 7-hexyl (C ₂₆ H ₅₄)	2-Hexyl-1-octanol (C ₁₄ H ₃₀ O)	1-Decanol, 2-hexyl (C ₁₆ H ₃₄ O)	40.280
Tetradecane, 2,6,10-trimethyl (C ₁₇ H ₃₆)	2-Hexyl-1-octanol (C ₁₄ H ₃₀ O)	1-Decanol, 2-hexyl (C ₁₆ H ₃₄ O)	42.481
Ethanol, 2- (octadecyloxy)- (C ₂₀ H ₄₂ O ₂)	2-Hexyl-1-octanol (C ₁₄ H ₃₀ O)	1-Decanol, 2-hexyl (C ₁₆ H ₃₄ O)	44.511
2-Hexyl-1-octanol (C ₁₄ H ₃₀ O)	Ethanol, 2- (octadecyloxy)- (C ₂₀ H ₄₂ O ₂)	1-Decanol, 2-hexyl (C ₁₆ H ₃₄ O)	46.412
2-Hexyl-1-octanol (C ₁₄ H ₃₀ O)	1-otadecane sulphonyl chloride (C ₁₈ H ₃₇ ClO ₂ S)	1-Decanol, 2-hexyl (C ₁₆ H ₃₄ O)	48.186

3.2. Control sample

The control sample was tested in the same conditions with other specimens and after extraction of the remaining hydrocarbons in the sludge sample, the hydrocarbon content was 252,500 (mg/kg dry sludge). Its reason can be the breakdown of some light hydrocarbons due to exposure to high autoclave pressure. In addition, there is the possibility

of entering a small amount of hydrocarbons into the liquid phase due to high pressure and temperature, which reduces the amount of solid phase hydrocarbons.

3.3. The effects of various materials adding to the oily sludge

3.3.1. Distilled water and oily sludge; sample 1

As shown in the Fig. 2, in the first week exists steep slope where is due to the presence of sufficient nutrients and easier access of microorganisms to linear and light hydrocarbons [23]. In addition, the native microorganisms in oily sludge and their previous adaptation to the environment have led to the removal of hydrocarbons quickly. The rotational speed of 130 rpm seems to be inadequate to provide the oxygen needed for the process, and in the last week, oxygen deficiency is a limiting factor, and the process of elimination has almost reached a steady state [11].

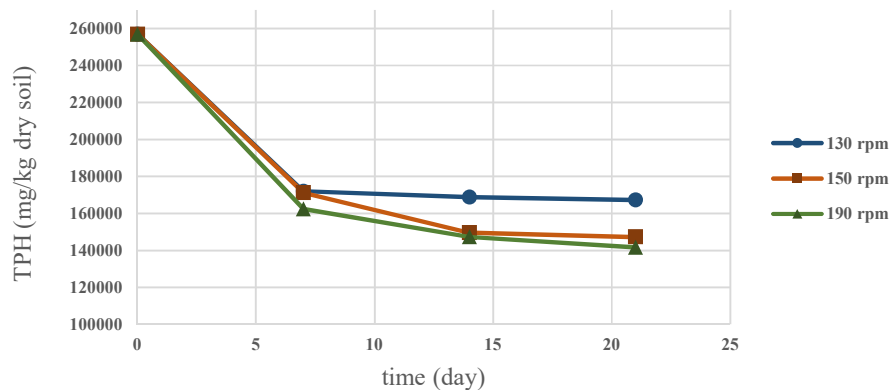


Fig. 2. Removal of TPH at different rotational speed for sample 1

3.3.2. Distilled water and Tween 80 and oily sludge; sample 2

Adding the Tween 80 surfactant reduces the contact between the phases of water-oil, solid-water and air-water, and reduces the tension between the oil compounds and the solid phase by reducing the surface tension and inter-surface elasticity. This phenomenon has increased the availability of microorganisms to oil and oil contaminants in oily sludge, which shows a higher removal rate than sample one. As shown in the Fig. 3, the amount of residual hydrocarbons in oily sludge has a decreasing trend over a period of three weeks. The sudden increase in gradient in the third week and speed of 190rpm is probability due to the release of nutrients and availability them for more activity of microorganisms in the last week [24].

3.3.3. Wastewater and oily sludge; sample 3

Wastewater with various nutrient sources improves the biodegradation process. In the third week (Fig. 4), despite the presence of nutrients and the continued growth of microorganisms, it can be argued that the continuation of the hydrocarbon decomposition process after the end of the period is also possible. As it turned out, at the highest rotational speed little effect on the increase of removal exist, which can be attributed to the fact that the mass transfer coefficient in this sample is constant and the increase in speed did not increase the mass transfer. There may also be

some anaerobic microorganisms in the sewage system that have been active in low-speed and oxygen deficiencies and have increased the removal of hydrocarbons.

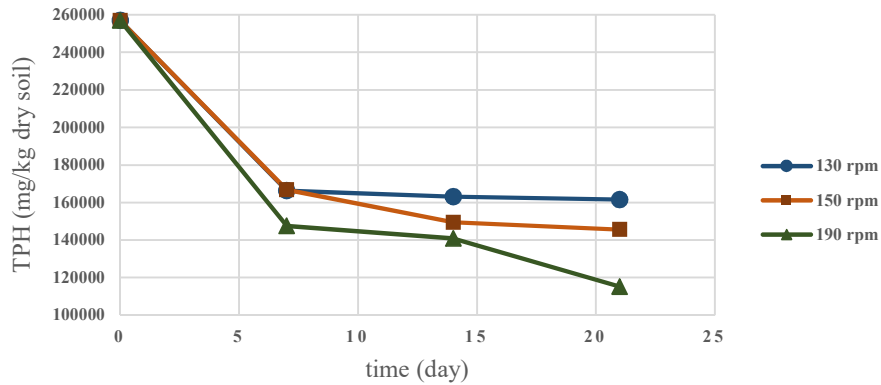


Fig. 3. Removal of TPH at different rotational speed for sample 2

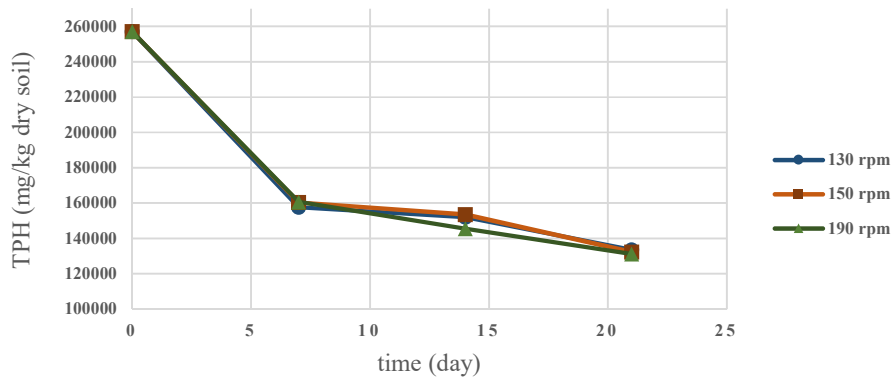


Fig. 4. Removal of TPH at different rotational speed for sample 3

3.3.4. Oily sludge and oily liquid; sample 4

Oily liquid along with oily sludge contains some indigenous microorganisms which effective in increasing the microbial population. The amount of hydrocarbon in this sample is very low. Because it is not possible for the organic phase to enter the water and the oil substance will not dissolve in the water. According to Fig. 5 the increase in the microbial population leads to an increase in their need for oxygen for growth and metabolism, and the speed of 130rpm is not responsible for providing sufficient amount of oxygen. As a result, the decrease in removal speed and biological activity can be confirmed at this rotation speed.

3.3.5. Wastewater, oily sludge and Tween 80; sample 5

By adding surfactants in order to increase the bioavailability of microorganisms, biodegradation is expected to improve. As shown in the Fig. 6, at the high rotational speed, Tween 80 performance has improved and the mass transfer from the organic phase to the aqueous phase has increased. The slope of the graph in the third week at 190 rpm has been abruptly increased, indicating the weakening of the micelles in the last week and breaking it at a high rotational speed. After the breakdown of the micelles, the nutrients present in it are available to other microorganisms in the environment and a greater hydrocarbon decomposition results.

The results obtained for the amount of hydrocarbons remaining in the sludge after the bioremediation process a reduction in the amount of hydrocarbons in all samples over a three-week process period. Also, due rotational speed, and as well as the by supplying oxygen to the native microorganisms in the oily sludge through aeration and with access to nutrient source through mixing, biodegradation activity increases.

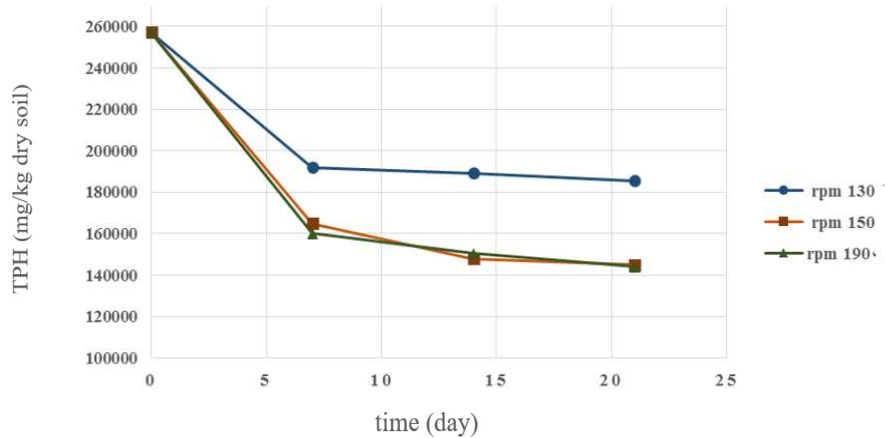


Fig. 5. Removal of TPH at different rotational speed for sample 4

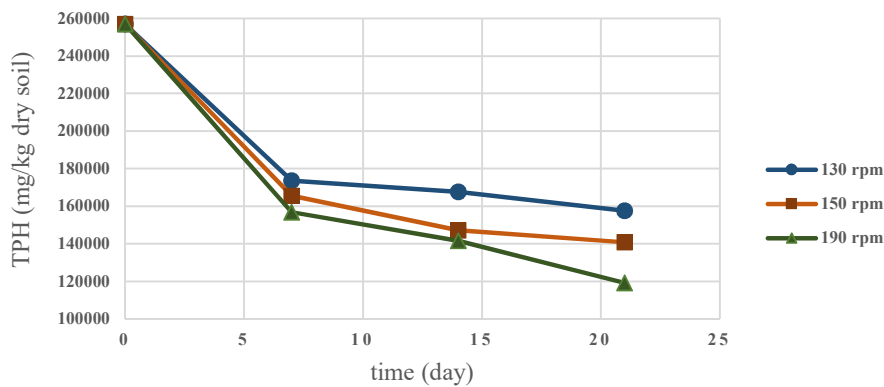


Fig. 6. Removal of TPH at different rotational speed for sample 5

3.3.6. Oily sludge, oily liquid and Tween 80; sample 6

As can be seen from the Fig. 7, adding Tween 80 to the petroleum liquid along with oily sludge has increased the efficiency of bioremediation compared to the state without surfactant. Due to the increase in the microbial population and the availability of pollutants and nutrients, the need for oxygen increases and more decomposition is observed at a higher stirring speed. According to data, it is clear that the sample with surfactant, speed of 130 rpm was not responsible for supplying the oxygen needed by the population of microorganisms and the process of hydrocarbon decomposition did not increase much in the last week.

3.3.7. Wastewater, oily sludge and oily liquid; sample 7

The simultaneous use of sewage and petroleum liquid along with sludge to create a slurry phase in the microbial population and nutrients in the sample 6. According to the Fig. 8, due to the increase in microbial population and

the need for more oxygen, the use of higher speeds has been more successful and has increased hydrocarbon removal.

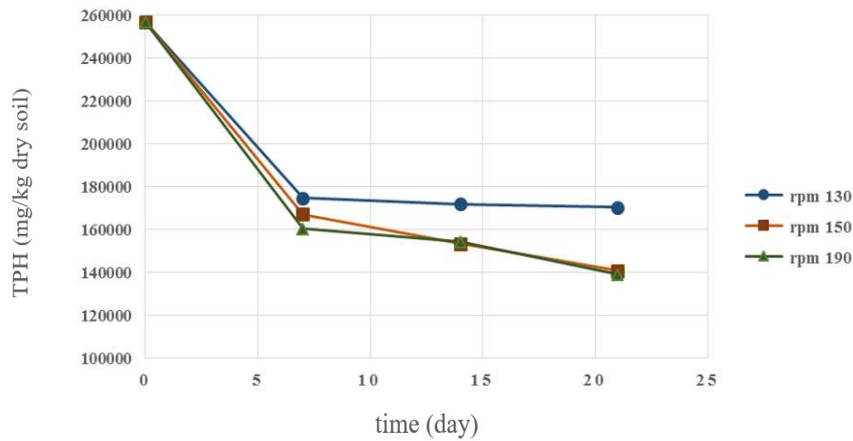


Fig. 7. Removal of TPH at different rotational speed for sample 6

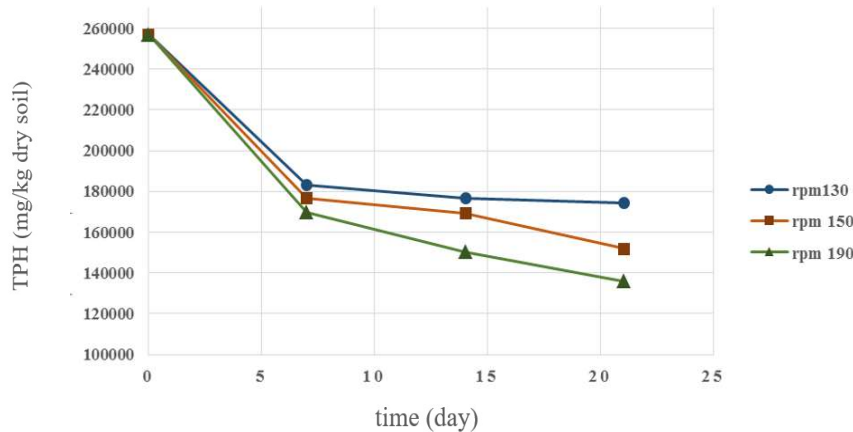


Fig. 8. Removal of TPH at different rotational speed for sample 7

3.3.8. Wastewater, oily sludge, oily liquid and Tween 80; sample 8

The combination of all three materials together; wastewater, Tween 80, and petroleum liquid with oily sludge makes carbon and nitrogen sources and pollutants available. As it is revealed in Fig. 9, the decreasing trend of the amount of hydrocarbon is maintained during the process time, but contrary to the expectation, this sample has almost less removal than other samples, which can be concluded that it is due to the increase in the population of microorganisms and the supply of the source. A large amount of carbon and a high availability of hydrocarbons have created an inhibition state and a lack of oxygen has disrupted the bioremediation performance.

3.4. Percentage of removal of the TPH

By comparing the obtained values for the percentage of total hydrocarbon removal in each sample at different speeds of rotation (Fig. 10), it seems that increasing the reactor agitator speed improves biodegradation and increases the removal of hydrocarbons. Surfactants act with two mechanisms of particle stimulation and dissolution of oil compounds in water. The mechanism of particle stimulation acts as a reduction in surface and intermediate surface tension of phases, and the dissolution mechanism results in the introduction of pollutants into the lipophilic section

of the micelles [25]. Ultimately, the use of Tween 80 is more effective at high speeds of rotation and mixing. Wastewater samples have been well removed at all three speeds. This surfactant first dissolves the nutrients in the wastewater and make them available for microorganism then absorbs oil contaminants. Therefore, wastewater samples and Tween 80 have less removal percentages than wastewater-only specimens. In addition, the use of Tween80 as a carbon source by microorganisms may be another reason for the obtained result [26].

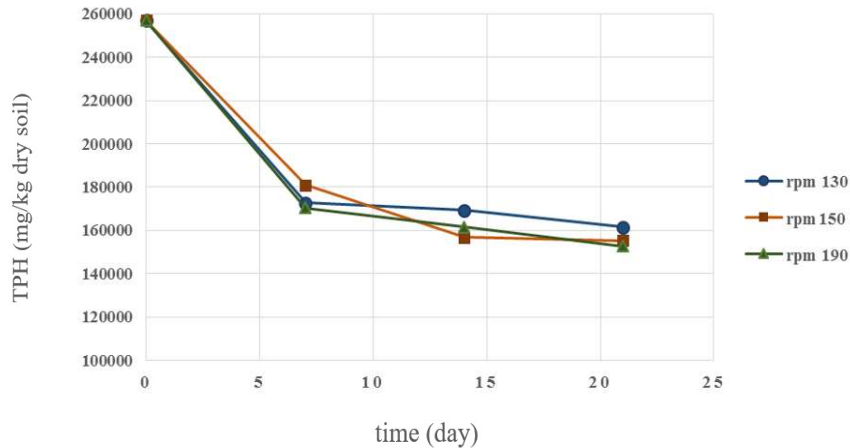


Fig. 9. Removal of TPH at different rotational speed for sample 8

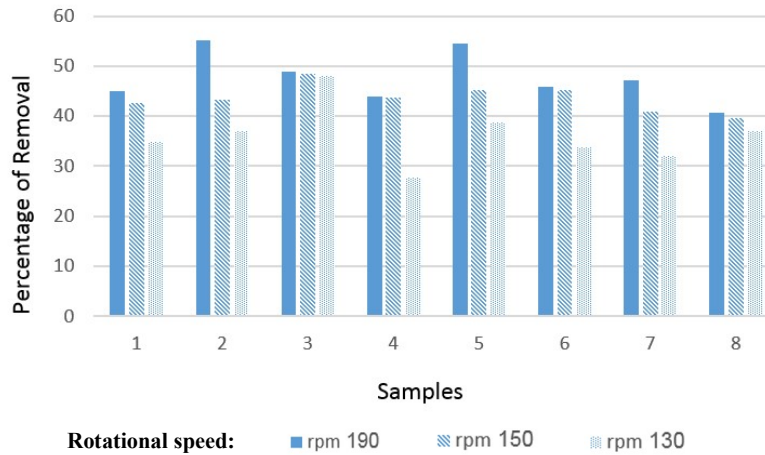


Fig. 10. hydrocarbon removal percent of the samples at different rotational speed

3.5. Chromatogram comparison before and after the process

Determining the amount of hydrocarbons in the gas chromatography (GC) test is performed by calculating the area under the curve drawn by the analysis system and comparing it with the area under the prototype curve (including the complete set of hydrocarbons available). By observing the results of the gas chromatography test taken for all specimens in the last week (Fig. 11), the accuracy of the results obtained with dichloromethane extraction was proved.

3.6. Investigation of liquid phase hydrocarbons

One specimen (wastewater sample) was analyzed by gas chromatography/mass spectrometry (GC mass) after 21 days of biodegradation (Fig. 12). By calculating the approximate hydrocarbon range, the TPH content in the water is obtained at a maximum of 1.5%, which is almost negligible. As shown, the compounds in the aqueous phase of this sample are acidic compounds that indicate the biological activity of the samples and remove hydrocarbons and convert them into less harmful intermediates.

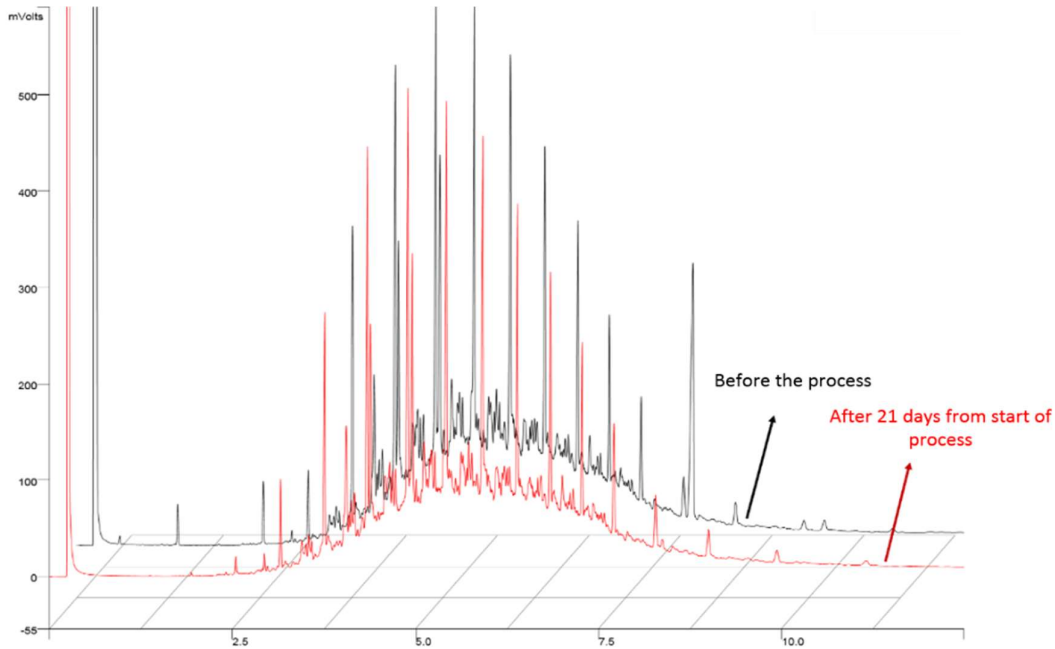


Fig. 11. Comparison of the gas chromatography test for the solid phase of sample 3 before and after the process

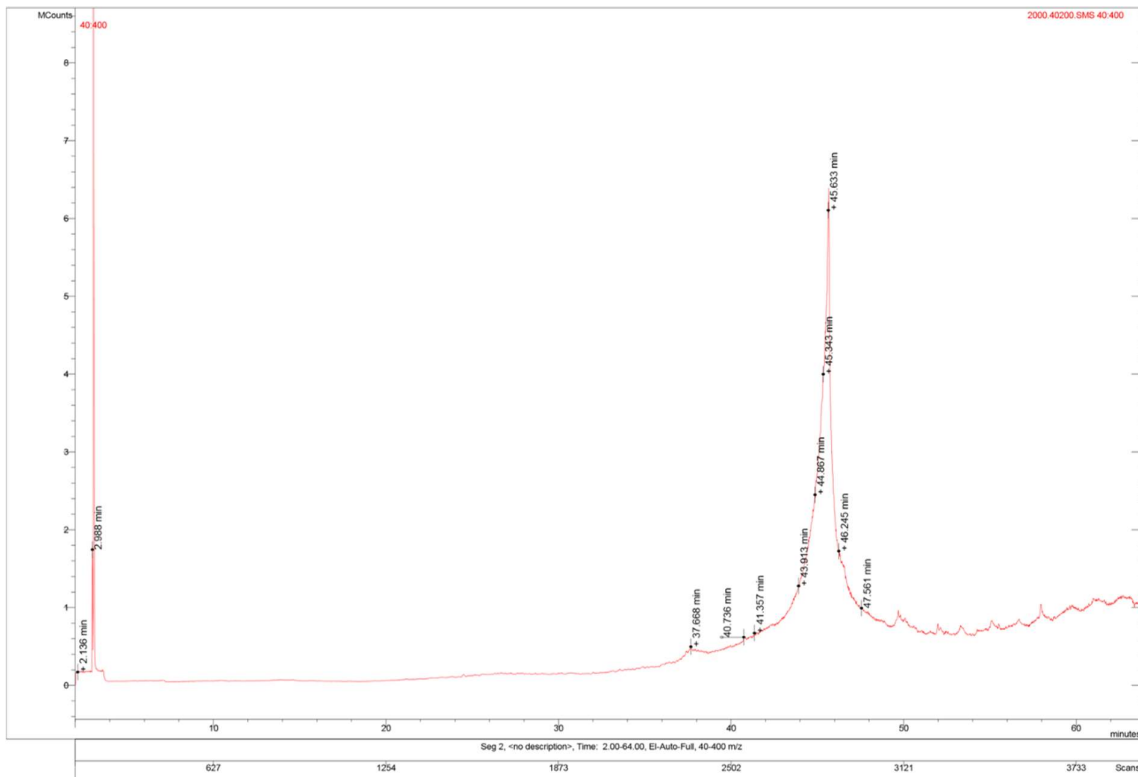


Fig. 12. Gas chromatogram with mass spectrometry of liquid phase after bioremediation process

3.7. pH of the samples in the liquid phase

The biological activity of microorganisms, Table 6, the pH of the samples is obtained after the end of the biodegradation process, and all the samples are in the acidic range. The lowest pH value is related to the rotational speed of 190. This is due to the better performance of microorganisms in this sample. However, acidity in the aqueous environment of all samples indicates the biological activity.

Table 6. pH amount in liquid phase

Samples	130 rpm	150 rpm	190 rpm
1	6.57	6.43	6.19
2	5.59	5.56	4.58
3	5.50	5.17	4.99
4	5.49	5.38	5.04

4. Conclusion

In this study, the biodegradation of oil sludge from the Sirjan gas storage reservoir in the slurry phase was investigated. The slurry phase increases the efficiency of microorganisms by increasing the mass transfer in the decomposition and degradation of hydrocarbons, and increases the removal rate in comparison with the solid phase. In order to optimize the bioremediation process urban sewage were used instead of water, and acceptable results were obtained. Adding wastewater to oily sludge to create a slurry phase has increased the removal and at different speeds given good results. Also, the use of surfactant Tween 80 in addition to reducing surface tension has increased the solubility of two phases and at high speeds of rotation, it has the highest removal percentage. In all samples, hydrocarbon removal has increased with increasing rotational speed. Considering the efforts made by all to reduce water consumption and reduce the cost of waste management, the use of wastewater instead of water is recommended to create a slurry phase and bioremediation of oil sludge. Besides, the use of petroleum liquid with sludge in creating slurry is not suitable and has shown little effectiveness. Of course, it may be more economical than using distilled water. Furthermore, the highest removal percentage was created at presence of Tween 80 and wastewater.

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