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Biodegradation of Aliphatic Hydrocarbons by Bacteria Isolated from Khor Al-Zubair Channel, Southern Iraq

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Abstract Four bacterial strains capable of using crude oil as the sole carbon source were isolated from Khor Al-Zubair channel, southern Iraq. The isolates were identified as *Vibrio vulnificus*; *Brevundimonas diminuta/vesicularis*; *Ochrobactrum anthropic* and *Sphingomonas paucimobilis* by the Vitek II system. The bacteria grew aerobically in liquid culture containing four concentrations (0.25%, 0.5%, 1% and 2% v/v) of crude oil at 30°C for a seven day period. The components of crude oil degraded by the isFolates were determined by capillary gas chromatography. *Vibrio vulnificus* was the most effective bacteria to degrade crude oil, especially in concentration 2% (93.05%). Meanwhile, *Ochrobactrum anthropi* was the lowest (9.63%) in concentration 0.25%.
Keywords Khor Al-Zubair; Bacteria; Vitek 2 system; Gas Chromatography

Introduction

The constituents of crude oil can be classified to four fractions: saturates aromatics, resins and asphaltenes. Each of these fractions contains a large number of compounds. Saturates is considered the major constituents of crude oil according to their chemical structures into alkanes (paraffins) and cycloalkanes (naphthenes). Aromatic hydrocarbons may have one or more aromatic rings with or without alkyl substitution(s). While, both the resin and asphaltene fractions do not contain hydrocarbon polar compounds. The elements present in resins and asphaltenes, in addition to carbon and hydrogen, are trace amounts of nitrogen, sulfur and/or oxygen (Harayama et al., 1999).

Petroleum hydrocarbons are the most common environmental pollutants in the world and oil spills pose a great hazard to terrestrial and marine ecosystems. Oil pollution may arise either accidentally or operationally whenever oil is produced, transported, stored and processed or used at sea or on land. Oil spills are a major menace to the environment as they severely damage the surrounding ecosystems (Head et al., 2006; Emtiazi et al., 2009).

Xenobiotic pollutants like crude oil can be removed from the environment by Biodegradation mechanism of naturally occurred microorganisms, this is the basic and save process (Cappello et al., 2007).

Because of the immiscibility of hydrocarbons in water, the growth of microorganisms on hydrocarbons is a specific problem. Biosurfactants are surface agents which increase the cell-substrate adhesion, that leads to emulsify hydrocarbons in solution, then the surface tension is reduced by biosurfactants due to its accumulation at the immiscible fluids increasing the insoluble compounds surface area which leads to rasing bioavailability and then hydrocarbons biodegradation (Batista et al., 2006).

The essential crude oil components are alkanes (Van Beilen et al., 2003). Some marine bacteria have the ability to biodegradation of alkanes and these bacteria are important for marine environment (Kohno et al., 2002).

Due to lack of functional groups as well as very low water solubility, aliphatic hydrocarbons exhibit boths, low chemical reactivity and bioavailability for microorganisms. However, some microorganisms possess the metabolic capacity to use these compounds as carbon and energy sources for their growth (Berthe-Corti and Fetzner, 2002).

The aim of this study was to isolate bacteria played important role in the process of bioremediation of oil-contaminated marine environments.

1 Materials and Methods

1.1 Water sampling

Samples collected from five stations at two depths from Khor Al-Zubair channel, southern Iraq (Figure 1). Sterile glass container (500 ml) used to collection for water samples and kept in ice boxes till reaching the laboratory.

St.		coordinations
1	N	30°11'33.82"
	E	47°53'24.51"
2	N	30° 9'0.41"
	E	47°54'4.83"
3	N	30° 6'42.96"
	E	47°55'26.92"
4	N	30° 5'19.06"
	E	47°56'52.26"
5	N	30° 3'30.55"
	E	47°57'30.74"

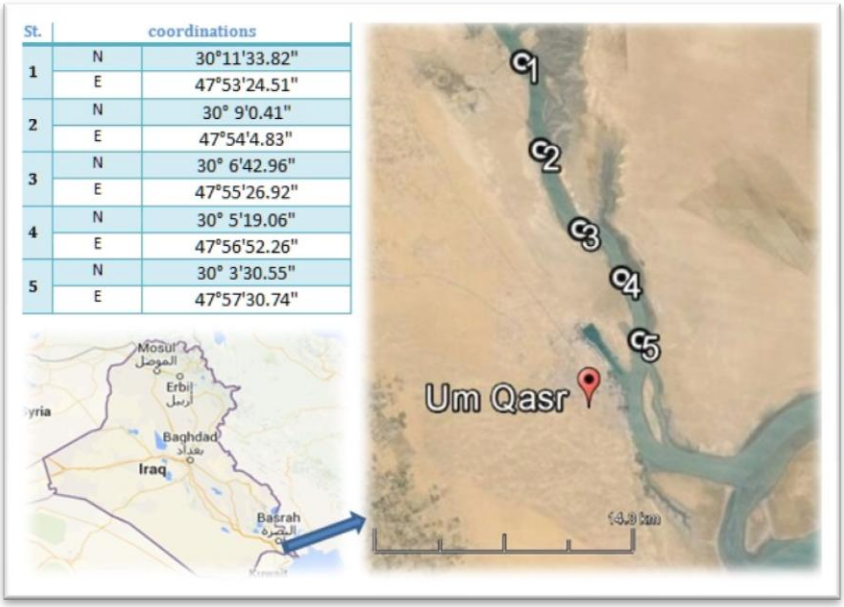

Figure 1 Sampling sites

1.2 Isolation and identification of bacterial species

One ml of every sample was cultured in a conical flask containing 100 ml mineral salts medium (MSM), which composed of 0.3 gm KCl, 1.0 gm K_2HPO_4, 0.5 gm KH_2PO_4, 0.01 gm $FeSO_4.7H_2O$, 30.0 gm NaCl, 0.5 gm $MnSO_4.7H_2O$, 0.2 gm $CaCl_2$ and 1000 ml D.W. (Fujisawa and Murakami, 1980). Four concentrations of crude oil added separately to the medium as 0.25 ml, 0.5 ml, 1 ml and 2.0 ml (Provided by Al-Shua'aba Refinery). Decimal dilutions of 7 days grown culture was cultivated at 30°C for 24 h. Nutrient agar (Hi media- India) with 30 ppt sodium chloride and marine agar (Difco, USA) were used to isolate *Vibrio vulnificus; Brevundimonas diminuta/vesicularis; Sphingomonas paucimobilis* and *Ochrobactrum anthropic* which identified by the Vitek II system (VK2C8300, USA).

1.3 Degradability study of crude oil

One ml of broth culture of each bacteria was incubated separately in 250 ml Erlenmeyer flasks containing 50 ml of MSM at 20 FID C for 7 days with shaking at 120 rpm using a cooling incubator shaker (Germany Sartorius Stidem-Certomat) (Al-Sulami et al., 2014). All the experiments were carried out in two duplicates, and the residual crude oil was estimated after 7 days.

1.4 Extraction of residual crude oil

Liquid-liquid extraction method was used for extraction of residual crude oil (Adebusoye et al., 2007),by using separating funnel,the aqueous phase is removed while the residual oil, dried in the oven at 40°C to eliminate CCl_4.

The separation column (length 25 cm, 3 cm) was used to separate the aliphatic fraction as described by Farid (2006), the remaining oil dissolved in n-hexane (25 ml), and poured in the separation column and drawn off the aliphatic part in 50 ml beaker as well as the control vials similarly, GC technique (Agilent Chem Station) used to estimate n-alkanes.

2 Results

A total of 65 bacterial isolates of gram negative fermentative and non-fermentative rods was investigated. Fifty six (86.15%) isolates were correctly identified to the species level, and 9 (13.84%) isolates were not identified. The direct-identification, reporting time of the Vitek II was 4.5- 10 h after incubation (Table 1).

Table 1 Identified bacteria using Vitek 2

Organism	Correctly identified%	Not identified%
V. vulnificus	15 (98)	
Sphingomonas paucimobilis	25 (95)	9
Brevundimonas diminuta/vesicularis	8 (91)	
Ochrobactrum anthropic	8	
Total	56	9

Four genera were isolated from Khor Al-Zubair channel, southren of Iraq and identified using the Vitek II system and investigated their ability to degrade crude oil in four concentrations.

Table 2 shows the percentage of degradation of crude oil for each genus. *V. vulnificus* was the most effective bacteria to degrade crude oil, especially in concentration 2% (93.05%). Meanwhile, *Ochrobactrum anthropi* was the lowest (9.63%) in concentration 0.25%.

Table 2 Degradation percentage

Bacterial types	Degradation percentage of crude oil (Aliphatic fraction)			
	0.25%	0.5%	1.0%	2.0%
Vibrio vulnificus	86.49%	83.87%	65.08%	93.05%
Brevundimonas diminuta/vesicularis	64.61%	81.63%	89.82%	69.15%
Ochrobactrum anthropic	9.63%	51.64%	68.03%	ND
Sphingomonas paucimobilis	79.19%	58.31%	78.19%	67.00%

The concentrations of normal-alkanes located between C9 and C28 with isopernoids-pristine and phytane were calculated by comparing with standard solutions. Figure 2 shows gas chromatography results of n-alkanes of control sample (0.25%). Biodegradation of n-alkanes by *V. vulnificus* after 7 days were shown in the results of gas chromatography (Figure 3) and the biodegradation percentage was 86.49% (Table 1). Figure 4 shows the gas chromatography results of crude oil incubated with *Brevundimonas diminuta/vesicularis* and the biodegradation percentage was 64.61% (Table 1). While Figure 5 and Figure 6 show the gas chromatography results of n-alkanes of crude oil incubated with *Ochrobactrum anthropic* and *Sphingomonas paucimobilis* and the biodegradation percentage were 9.63% and 79.19% respectively.

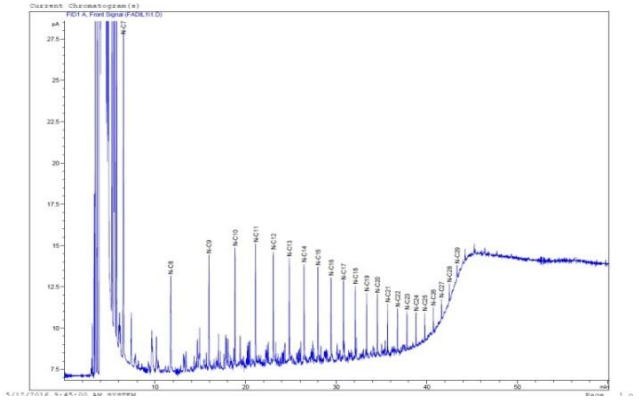

Figure 2 Crude oil (aliphatic fraction) 0.25%

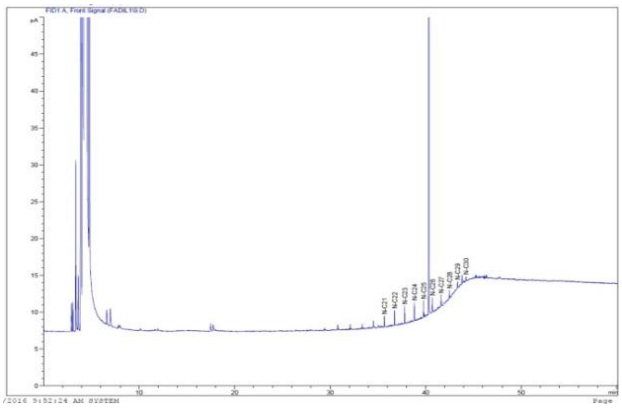

Figure 3 Crude oil 0.25% with *V.vulnificus*

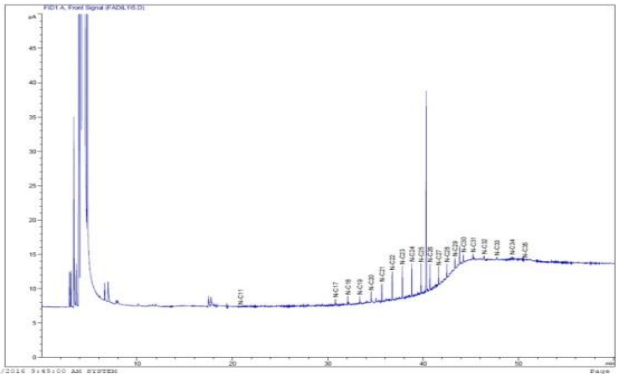

Figure 4 Crude oil 0.25% with *Brev.dimi./vesicu*

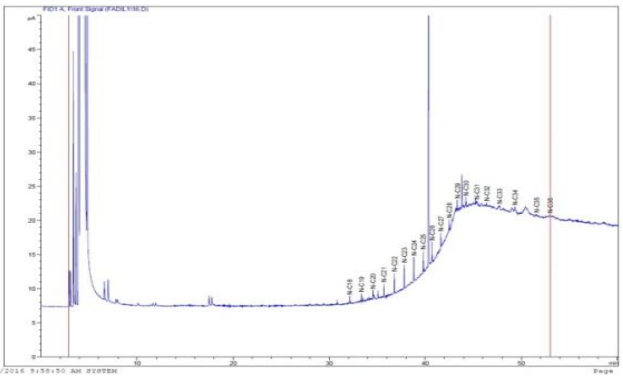

Figure 5 Crude oil 0.25% with *Ochrobac.anthro*

Figure 7 shows gas chromatography results of n-alkanes of control sample (0.5%). Whereas Figure 8, Figure 9, Figure 10 and Figure 11 show the gas chromatography results of n-alkanes of crude oil incubated with *V. vulnificus, Brevundimonas diminuta/vesicularis, Ochrobactrum anthropic* and *Sphingomonas paucimobilis* and the biodegradation percentage were 83.87%, 81.63%, 51.64% and 58.31% respectively (Table 1).

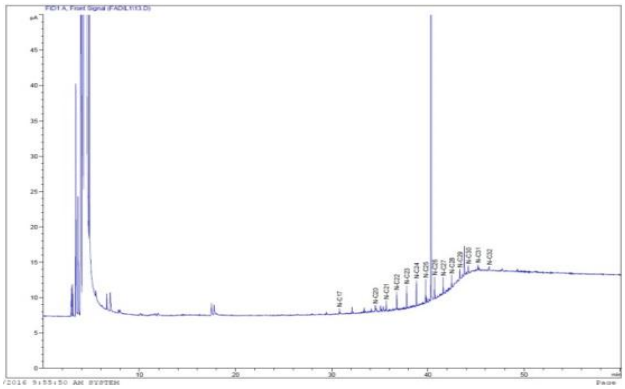

Figure 6 Crude oil 0.25% with *Sphmon.paucimobilis*

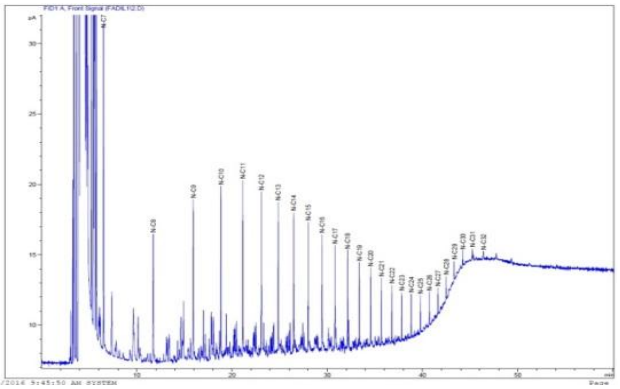

Figure 7 Crude oil (Aliphatic fraction) 0.5%

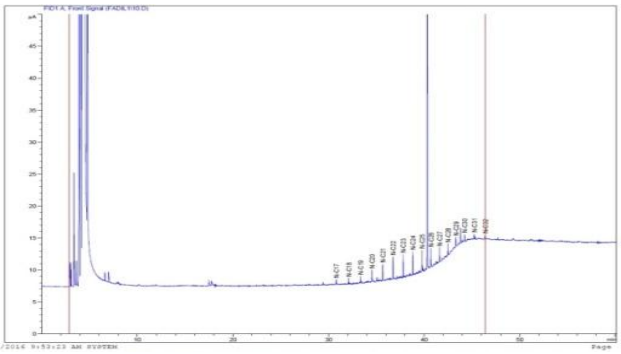

Figure 8 Crude oil 0.5% with *V.vulnificus*

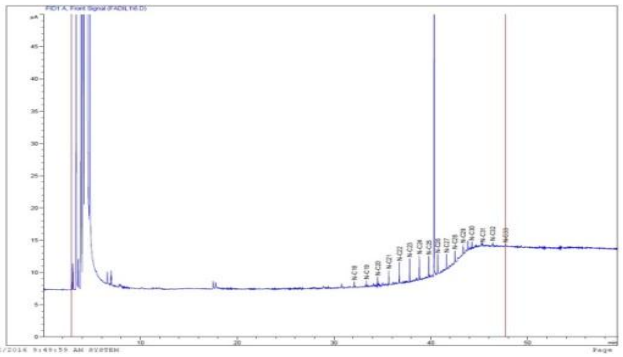

Figure 9 Crude oil 0.5% with *Brev.dimi./vesicu*

Figure 12 shows gas chromatography results of n-alkanes of control sample (1%). Whereas Figure 13, Figure 14, Figure 15 and Figure 16 show the gas chromatography results of n-alkanes of crude oil incubated with *V. vulnificus, Brevundimonas diminuta/vesicularis, Ochrobactrum anthropic* and *Sphingomonas paucimobilis* and the biodegradation percentage were 65.08%, 89.82%, 68.03% and 78.19% respectively (Table 1).

Figure 17 shows gas chromatography results of n-alkanes of control sample (2%). Whereas Figure 18, Figure 19 and Figure 20 show the gas chromatography results of n-alkanes of crude oil incubated with *V. vulnificus, Brevundimonas diminuta/vesicularis, and Sphingomonas paucimobilis* and the biodegradation percentage were 93.05, 69.15% and 67.00% respectively (Table 1). While *Ochrobactrum anthropic* not determined.

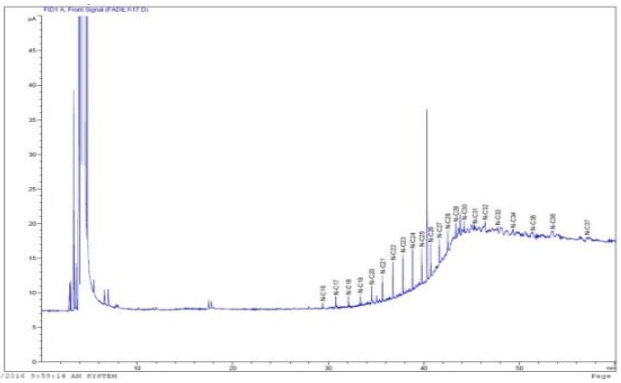

Figure 10 Crude oil 0.5% with *Ochrobac.anthropi*

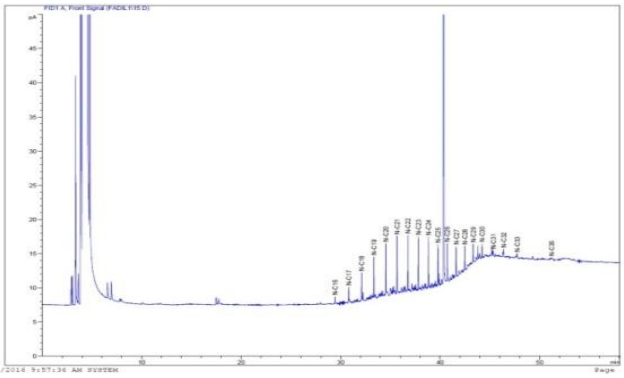

Figure 11 Crude oil 0.5% with *Sphmon.paucimobilis*

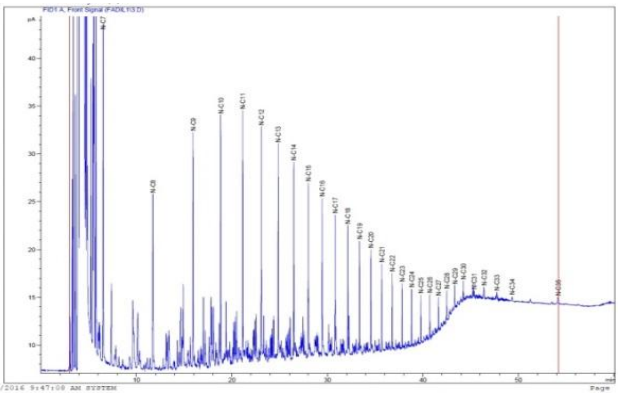

Figure 12 Crude oil (Aliphtic fraction) 1.0%

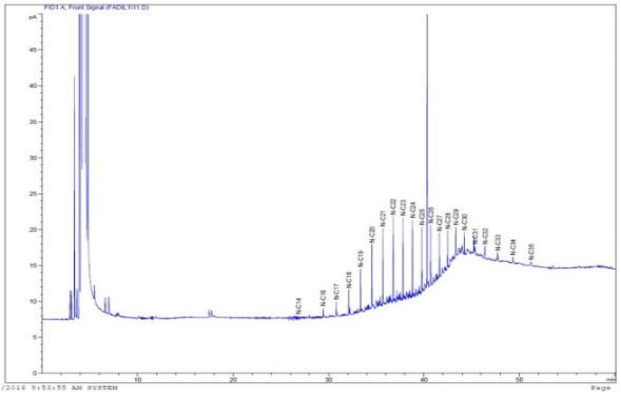

Figure 13 Crude oil 1.0% with *V.vulnificus*

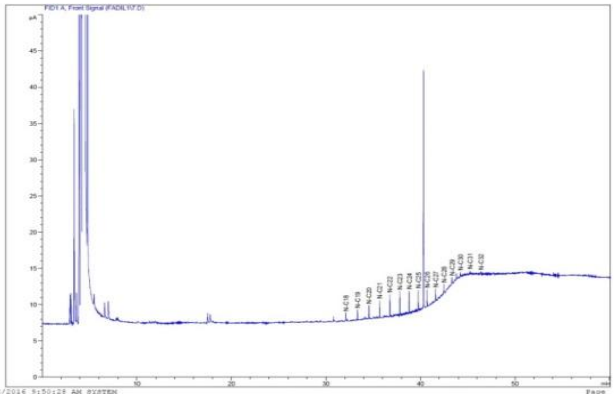

Figure 14 Crude oil 1.0% with *Brev.dimi./vesicu*

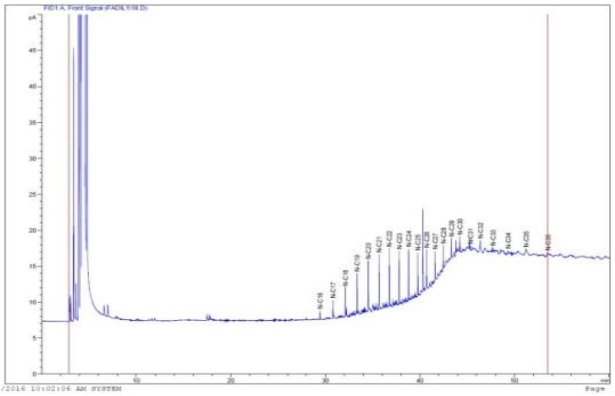

Figure 15 Crude oil 1.0% with *Ochrobac.anthropi*

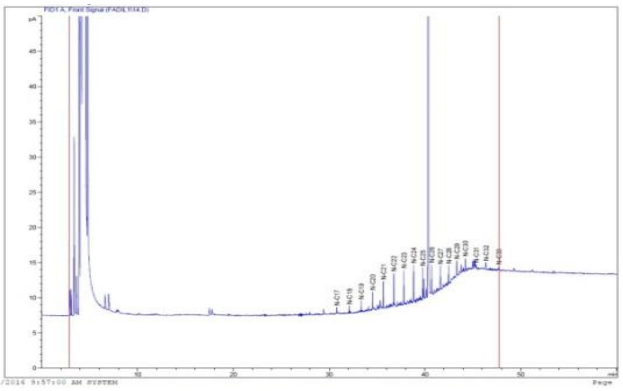

Figure 16 Crude oil 1.0% with *Sphmon.paucimobilis*

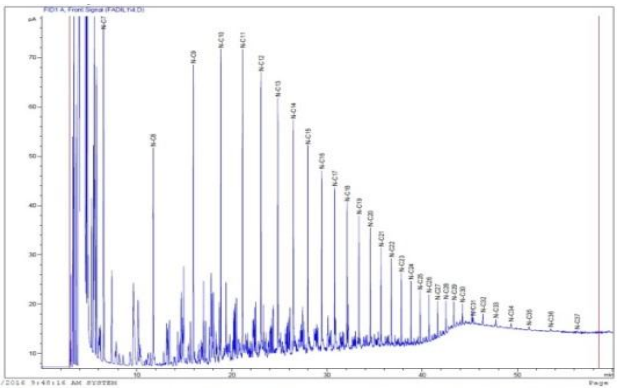

Figure 17 Crude oil (Aliphtic fraction) 2.0%

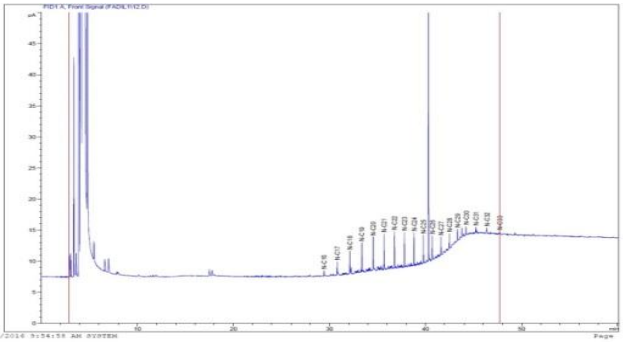

Figure 18 Crude oil 2.0% with *V.vulnificus*

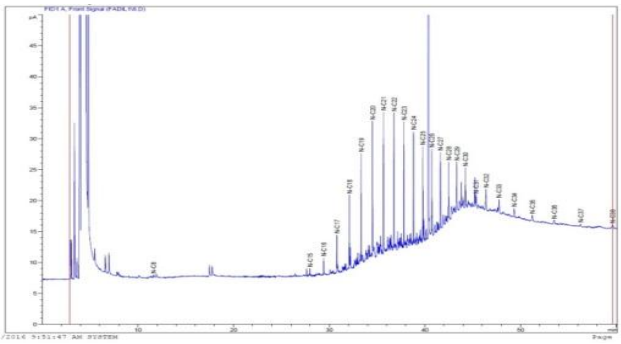

Figure 19 Crude oil 2.0% with *Brev.dimi./vesicu*

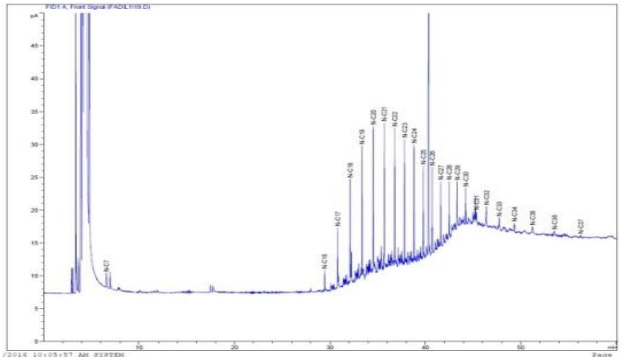

Figure 20 Crude oil 2.0% with *Sphmon.paucimobilis*

3 Discussion

The microorganisms which have the ability to degrade crude oil and use it as a sole carbon source are widely distributed in air, water and soil (Bello, 2007; Al-Sulami et al., 2014). Four genera have been identified as *Vibrio vulnificus; Brevundimonas diminuta/vesicularis; Ochrobactrum anthropic* and *Sphingomonas paucimobilis* from brackish water in Khor Al-Zubair, southern Iraq, using the VITEK II system. The VITEK II direct identification testing of gram negative bacilli had a correct identification rate of 86.15%, none of the results gave a wrong identification and nine (13.84%) were not identified. These bacteria have been participating in the process of crude oil degradation and exploited it as a source of carbon and energy, according to Kosaric (2000) three models of hydrocarbon transport of bacterial cells are considered, either by interaction of cells with more water soluble hydrocarbons, or by the direct touch of cells with big drops of hydrocarbon. During this process bacterial cells attach to the surface of hydrocarbon drops that are too bigger than cells; the substrate surface area availability for cell attachment is a limiting factor; production of biosurfactant by hydrocarbon utilizing bacteria cause the dispersion of hydrocarbon droplets in the aqueous environment and that way leads to increasing the surface area. And bacterial cells interact with particles of solubilized, microemulisifeid hydrocarbons. So, according to the type of organism, hydrocarbon uptake/degradation may occur in one of these mechanisms (Prabhu and Phale, 2003). The action of these mechanisms may include alteration in cell physiology, which will cause either to changes in the properties of cell surface as hydrophobicity or secretion compounds into the medium or a combination of both (Plaza et al., 2005). The results indicate that, these bacteria have the ability to attack all the hydrocarbon chains within seven days. Despite the fact that, many studies find, the microorganisms attack first, lower and higher hydrocarbon chains and those of middle length were attacked later in the course of incubation (Bello, 2007; Malik and Ahmed, 2012; Al-Sulami et al., 2014).

In the present study, four concentrations of crude oil were studied in liquid medium by axenic culture of four different genera. The results of gas chromatography showed that, there are fluctuations in the ability of bacteria to degrade crude oil (Table 2), but the isolates of *V. vulnificus* was the most effective strain has the ability to degrade crude oil, which reached 93.05% in concentration 2%, while *Ochrobactrum anthropi* was the lowest 9.63% in concentration 0.25%.

4 Conclusion

Four strains of bacteria have been isolated from brackish water and identified by the Vitek II system. The ability of these strains to uptake/degradation crude oil has been exploited. There is a fluctuation in their abilities to degrade hydrocarbon, this is may be related to the types of bacteria and type of mechanisms which used to degrade crude oil.

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