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Estimation of Bacterial Biodegradability of PAH in Khor Al-Zubair Channel, Southern Iraq

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Abstract Four bacterial strains were isolated from Khor Al-Zubair channel, southern Iraq based on a high growth rate on crude oil and on hydrocarbon degradation ability. The strains were preliminarily identified based on morphological observation, and by the Vitek II system. The isolates were identified as *Brevundimonas diminuta /vesicularis*, *Vibrio vulnificus*, *Sphingomonas paucimobilis* and *Ochrobactrum anthropic*. However, the ability of these strains to utilize crude oil (0.25%; 0.5%; 1% and 2% v/v) varied both in rates of utilization and in growth profiles. The components of crude oil degraded by the isolates were determined by capillary gas chromatography. *Sphingomonas paucimobilis* and *Vibrio vulnificus* were the most effective bacteria degrade PAH on seventh day incubation, especially in concentration 2.0 mg/l (97.39% and 84.23%) respectively, meanwhile, *Brevundimonas diminuta/ vesicularis* was highly effective degrade PAH (73.41% and 62.08%) in concentration 1.0 and 0.5 mg/l, respectively. On the other hand, *Ochrobactrum anthropi* was the lowest in all concentrations.
Keywords Khor Al-Zubair; Bacteria; Vitek II system; Gas chromatography

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds consisting of two or more aromatic rings, the USEPA has reported sixteen unsubstituted PAHs as main concern pollutants, eight of them may be human carcinogens (Yan et al., 2004). The concern is that some PAHs are toxic, carcinogenic or teratogenic. PAHs are a resource of carbon and energy and being exploited by some bacteria as growth substrates. The presence of PAH in the marine environment is largely attributed to oil spills, discharge and natural river infiltration, import or transfer of airflow. Therefore, global increased human activity has increased risks to the marine environment (Latimer and Zheng, 2003).

PAHs are present as small and large, depending on the number of rings. "Small" PAHs contain up to six condensed aromatic rings, whereas, "large" PAHs containing more than six aromatic rings, which are less soluble in water and less volatile (Kumar et al., 2011).

Although PAHs fact that undergo oxidation, photolysis, bioaccumulation, volatilization and adsorption in the environment, but have been microbial degradation and transformation as the key processes identified for the removal of pollutants (Zeng et al., 2010).

Biodegradation is a viable bioremediation technology for organic pollutants. It has identified that microorganism degrade ecological pollutant in various environments. Bioremediation uses the metabolic adaptation of microorganism to degrade contaminations. Bioremediation is a promising approach, as it employs microorganisms to degrade or remove different pollutants, such as organic compounds into harmless metabolites or mineralize the contaminants to CO_2 and water (Alexander et al., 1985).

The rate of biodegradation depends on pH, temperature, microbial population, oxygen, nutrient accessibility, chemical structure of the compound, the division of properties and cellular chemical transport in the growth medium (Singh and Ward, 2004). The aim of the present study was to isolate and identify new bacteria that have the ability to degrade polycyclic aromatic hydrocarbons.

1 Materials and Methods

1.1 Collection of samples

Samples were collected from Khor Al-Zubair channel, southern Iraq (Figure 1) from five stations. Water samples (500 ml) were collected at two depths in sterile glass bottle, all samples transferred to an ice box and transported to the laboratory.

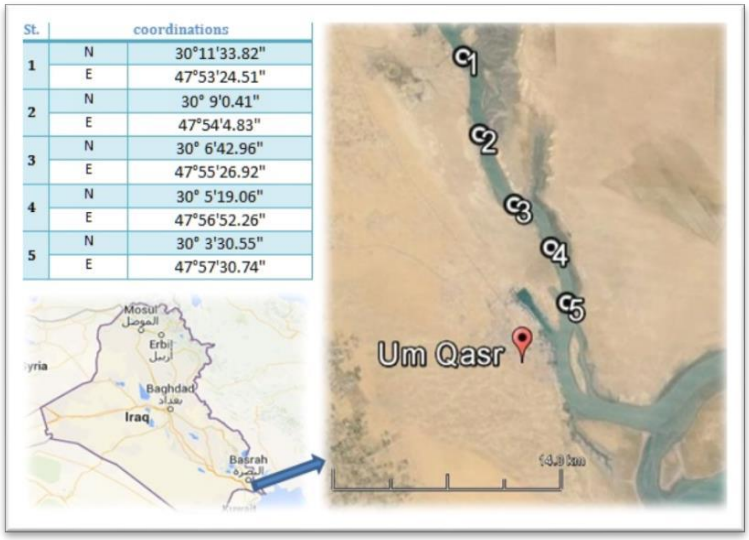

Figure 1 Sampling locations

1.2 Isolation and identification of bacteria

One milliliter of each sample was cultured in a conical flask containing 100 ml mineral salts medium (MSM), the composition of the MSM was 0.3 gm of KCl, 1.0 gm of K_2HPO_4, 0.5 gm of KH_2PO_4, 0.01 gm of $FeSO_4.7H_2O$, 30.0 gm of NaCl, 0.5 gm of $MnSO_4.7H_2O$, 0.2 gm of $CaCl_2$ and 1000 ml DW (Fujisawa and Murakami, 1980). Four concentrations of crude oil as 0.25 ml, 0.5 ml, 1 ml and 2.0 ml (Provided by Al-Shua'aba Refinery) were added separately to the medium. Decimal dilutions of 7 days grown culture was cultivated at 30 ℃.

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* and identified by the Vitek II system (VK2C8300, USA).

1.3 Degradation of PAH

One millilitre of broth culture of each bacterial isolate was incubated separately in 250 ml Erlenmeyer flask containing 50 ml of MSM at 20°C for 7 days at 120 rpm using a cooling incubator shaker (Al-Sulami et al., 2014). All the experiments were carried out in two duplicates, and the residual crude oil was performed after 7 days.

1.4 Gas chromatographic analysis of residual crude oil

The residual crude oil was extracted by liquid-liquid extraction, as described by Adebusoye et al. (2007). The aqueous phase was removed by separating funnel and the residual oil dried in the oven at 40°C to remove CCl_4.

The aromatic fraction was separated using separation column (25 cm length, 3 cm diameter) containing 8 gm of silica gel over a little amount of glass cotton (Farid, 2006). The residual oil dissolved in 25 ml of benzene and poured into the separation column and drawn off the aromatic fraction in 50 ml beaker. Control flasks were also extracted similarly, aromatic fraction hydrocarbons estimated by FID gas chromatography technique (Agilent Chem Station).

2 Results and Discussion

2.1 Identification of bacteria

Sixty five isolates of gram negative bacilli was isolated from Khor Al-Zubair channel southern Iraq, Fifty-six of them were identified correctly to the species level as *V. vulnificus*, *Sphingomonas aucimobilis*, *Brevundimonas diminuta/ vesicularis* and *Ochrobactrum anthropi*, while 9 strains were not identified. Direct identification, reporting time of the VITEK II was 4.5 to 10 hours after incubation (Table 1). Four species were isolated by the VITEK II system and their ability to degrade four concentrations of crude oil was examined.

Table 1 Identification of bacteria isolates by Vitek II

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

2.2 Degradation study of PAH

In the present study, four species were examined their capability to degrade 4 concentrations of crude oil. Table 2 shows the percentage of degradation of PAH for each genus. *Sphingomonas paucimobilis* was the most effective bacteria to degrade PAH (97.39%) especially in concentration 2.0 mg/l, while *Ochrobactrum anthropi* was the lowest (13.15%) in the same concentration. Figure 2, Figure 3, Figure 4 and Figure 5 show GC results of control samples (0.25%; 0.5%; 1% and 2%). *Brevundimonas diminuta/ vesicularis* showed high PAH degradation (73.41%) in concentration 1.0 mg/l and low degradation (25.66%) in concentration 2.0 mg/l (Table 2; Figure 6; Figure 7; Figure 8; Figure 9).

Table 2 Degradation percentage of PAH

Bacterial types	0.25 mg/l	0.5 mg/l	1.0 mg/l	2.0 mg/l
Brevundimonas diminuta/vesicularis	27.71%	62.08%	73.41%	25.66%
Vibrio vulnificus	64.03%	22.91%	42.18%	84.23%
Sphingomonas paucimobilis	33.76%	27.26%	31.22%	97.39%
Ochrobactrum anthropic	ND	19.00%	14.45%	13.15%

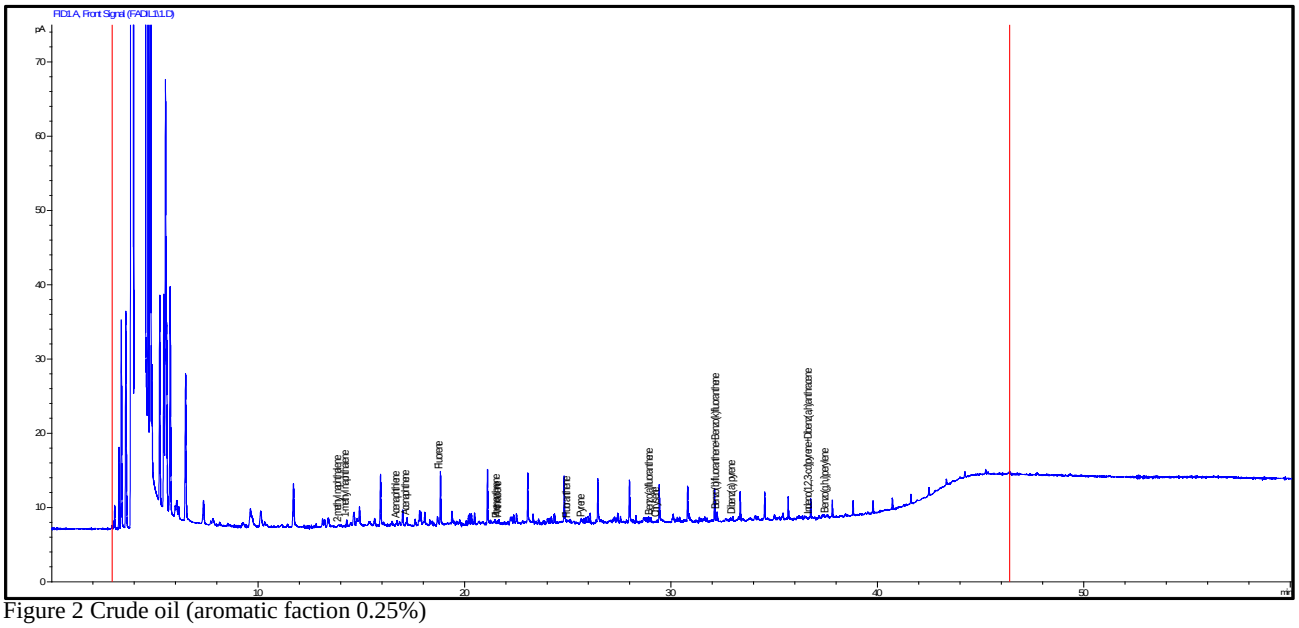

Figure 2 Crude oil (aromatic faction 0.25%)

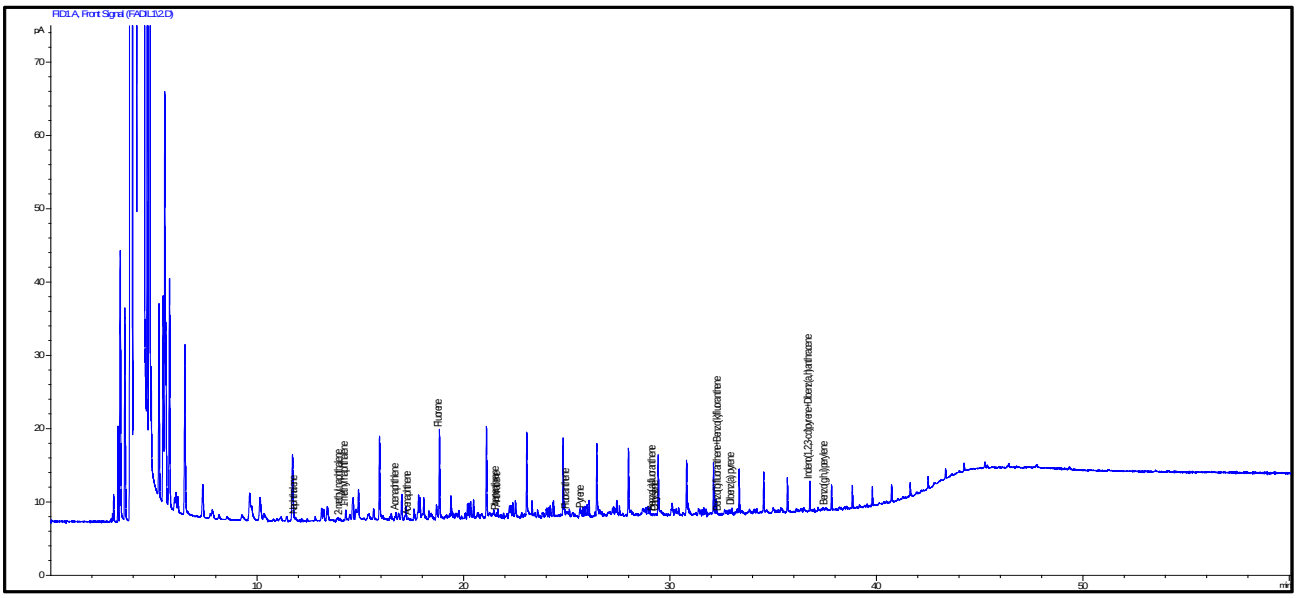

Figure 3 Crude oil (aromatic fraction 0.5%)

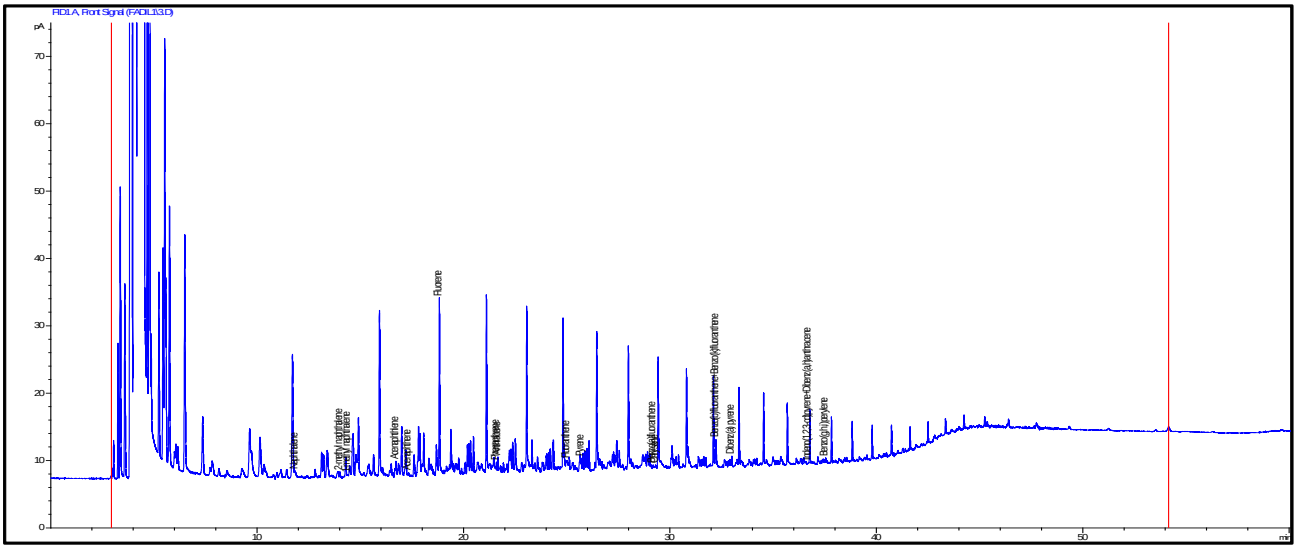

Figure 4 Crude oil (aromatic fraction 1.0%)

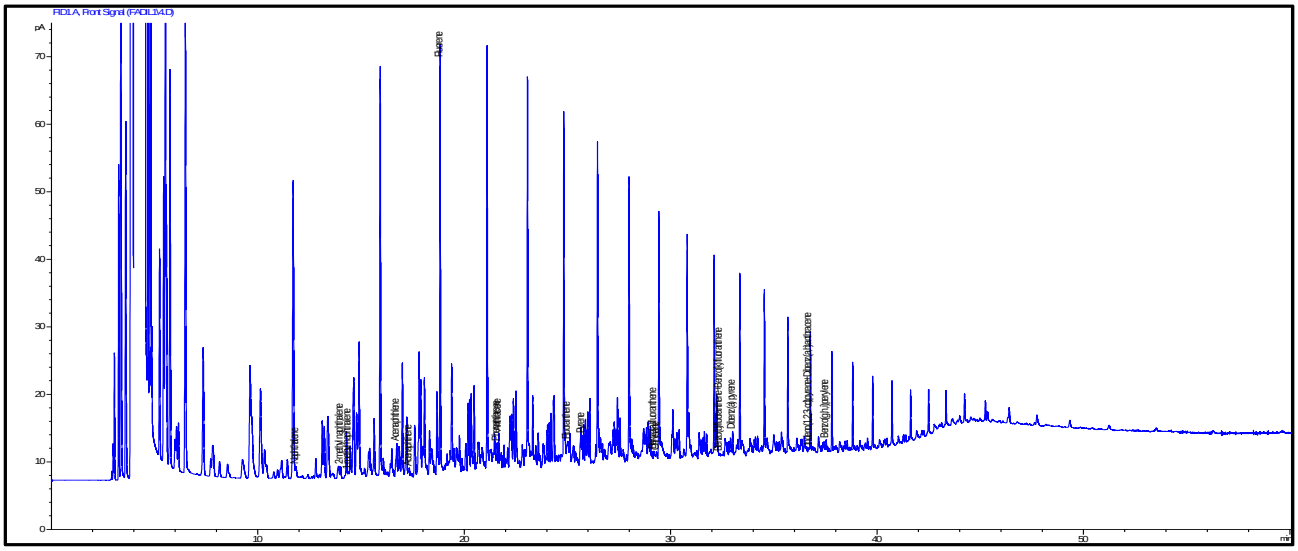

Figure 5 Crude oil (aromatic fraction 2.0%)

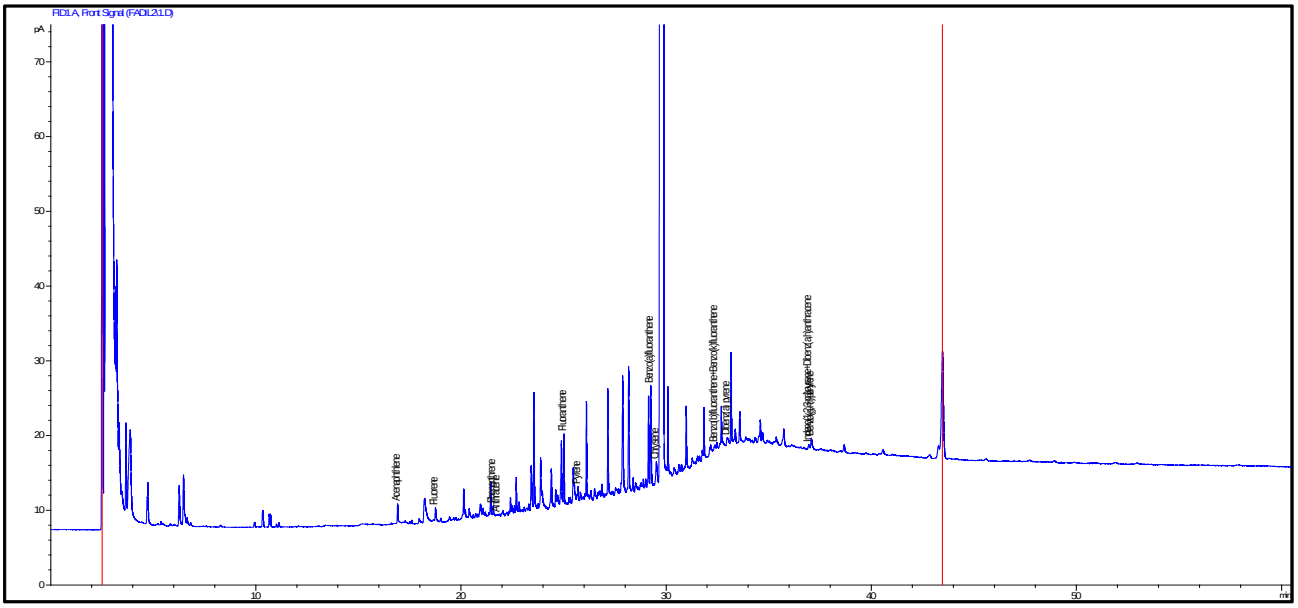

Figure 6 Crude oil 0.25% with *Brev. dimi./vesicu.*

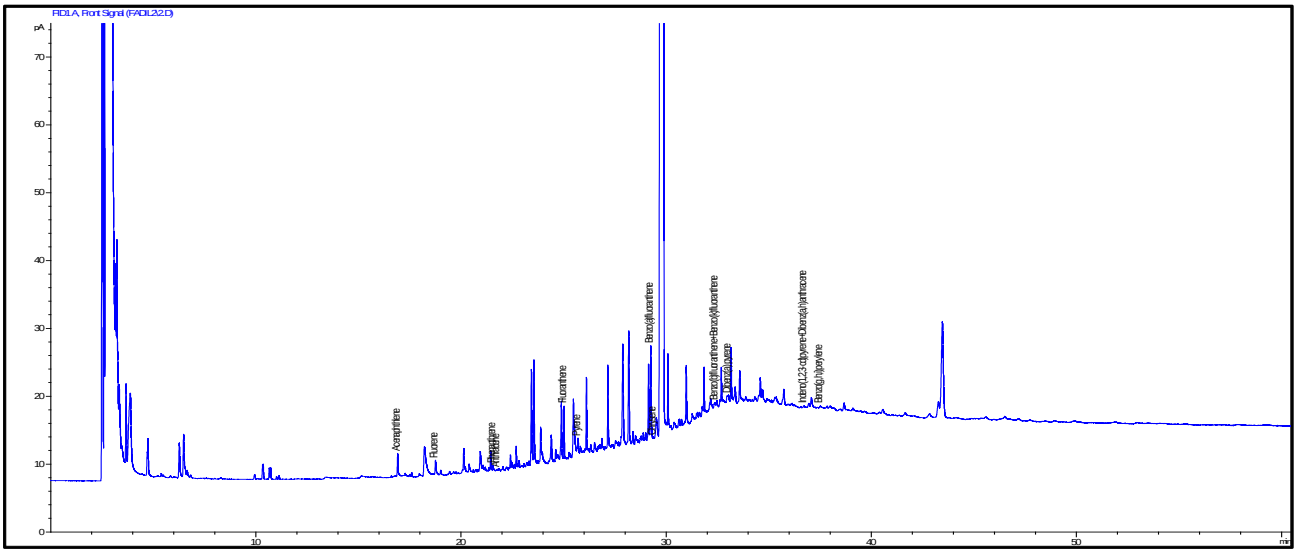

Figure 7 Crude oil 0.5% with *Brev. dimi./vesicu.*

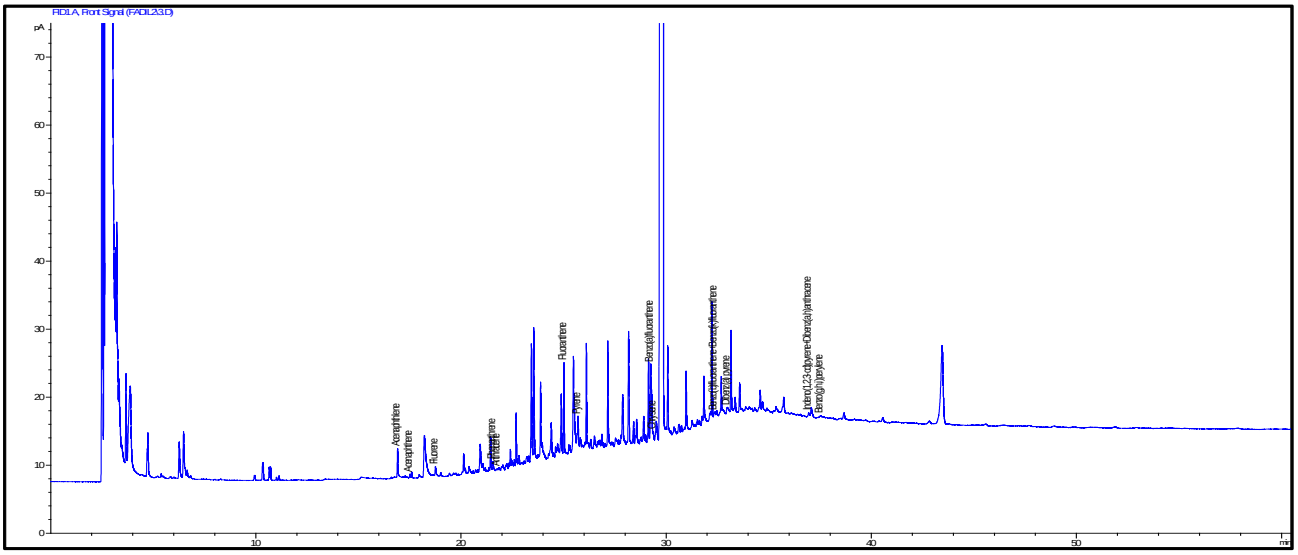

Figure 8 Crude oil 1.0% with *Brev. dimi./vesicu.*

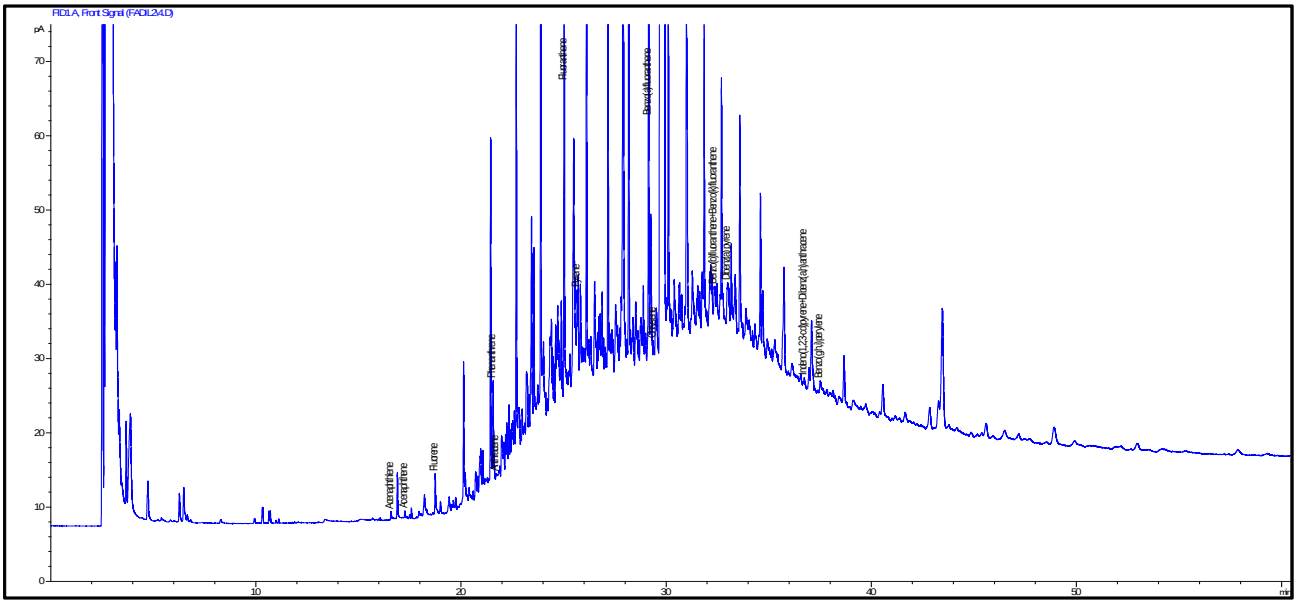

Figure 9 Crude oil 2.0% with *Brev. dimi./vesicu.*

On the other hand, *Vibrio vulnificus* showed high degradation percentage of PAH (84.23%) in concentration 2.0 mg/l and low degradation (22.91%) in concentration 0.5 mg/l (Table 2; Figure 10; Figure 11; Figure 12; Figure 13). The genus of *Vibrio* was first described as a degrading phenanthene organism from West and colleagues, who isolated large numbers of strains by spreading Chesapeake Bay samples onto complex media each with a superficial layer of phenanthrene (Okpokwasili et al., 1984; West et al., 1984).

Sphingomona paucimobilis showed high degradation percentage (97.39%) in concentration 2.0 mg/l and low degradation (27.26%) in concentration 0. 5 mg/l (Table 2; Figure 14; Figure 15; Figure 16; Figure 17). This is consistent with a report by Tao et al. (2007) who found that this bacterium has unique genes to degrade a wide range of PAH and related compounds. The gene is related from other Pseudomonas genera and reports so far in sequence homology and gene organization. Also Pinyakong et al. (2003a; 2003b) observed that, many sphingomonads degrade naphthalene, anthracene, and phenanthalene by common pathways were found in other gram-negative bacteria. In addition, many researchers (Kim et al., 1996; Feng et al., 1997; Romine et al., 1999; Ogram et al., 2000; Basta et al., 2004) found large plasmids in strains of *Sphingomonas xenobiotic* contributing to the degradation of PAH.

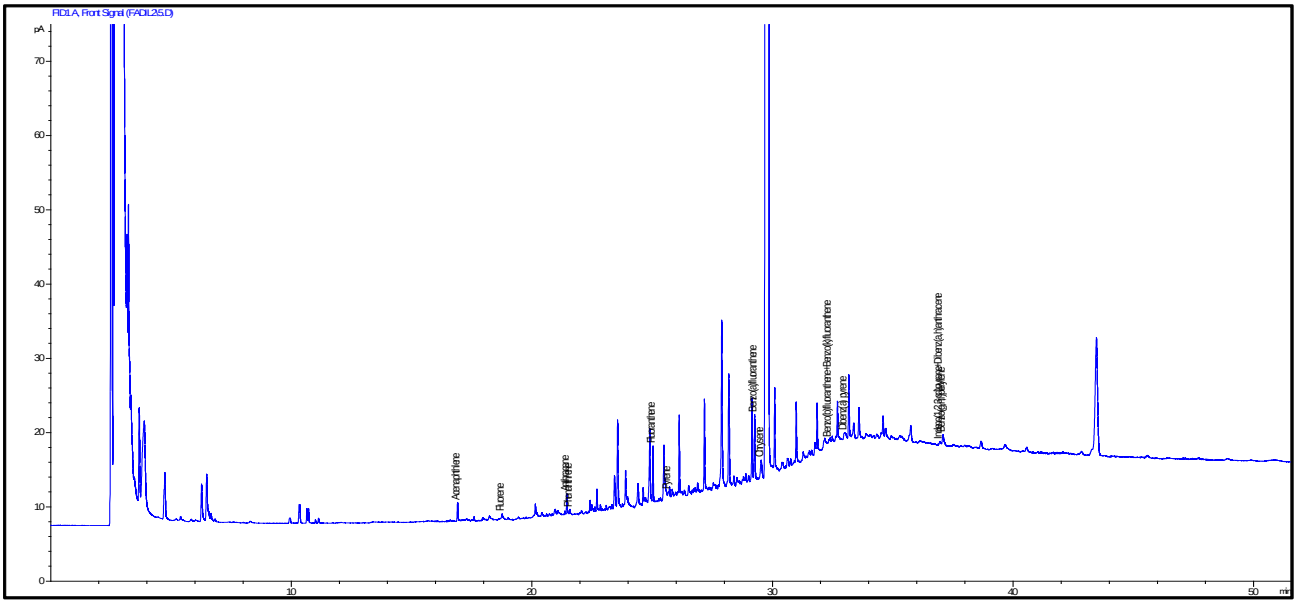

Figure 10 Crude oil 0.25% with *V. vulnificus*

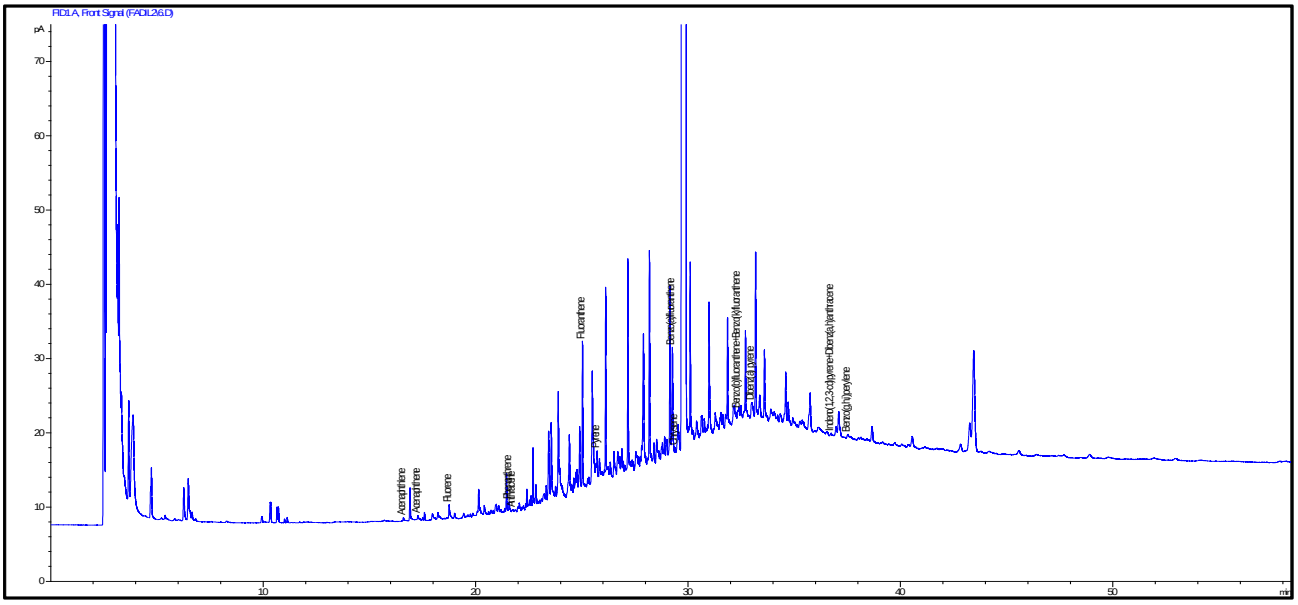

Figure 11 Crude oil 0.5% with *V. vulnificus*

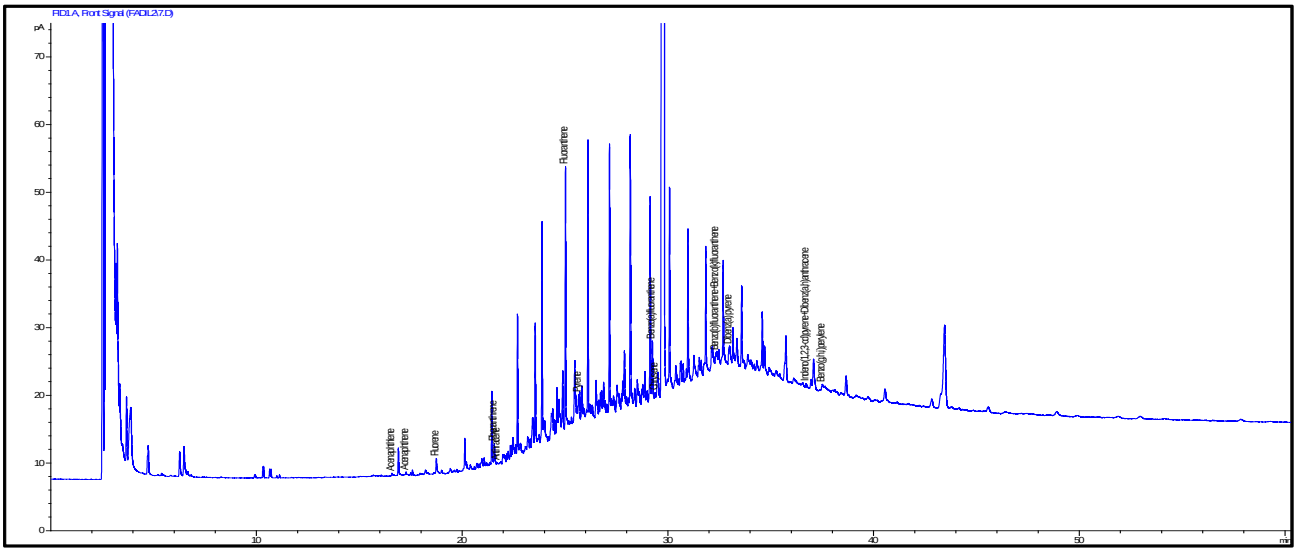

Figure 12 Crude oil 1.0% with *V. vulnificus*

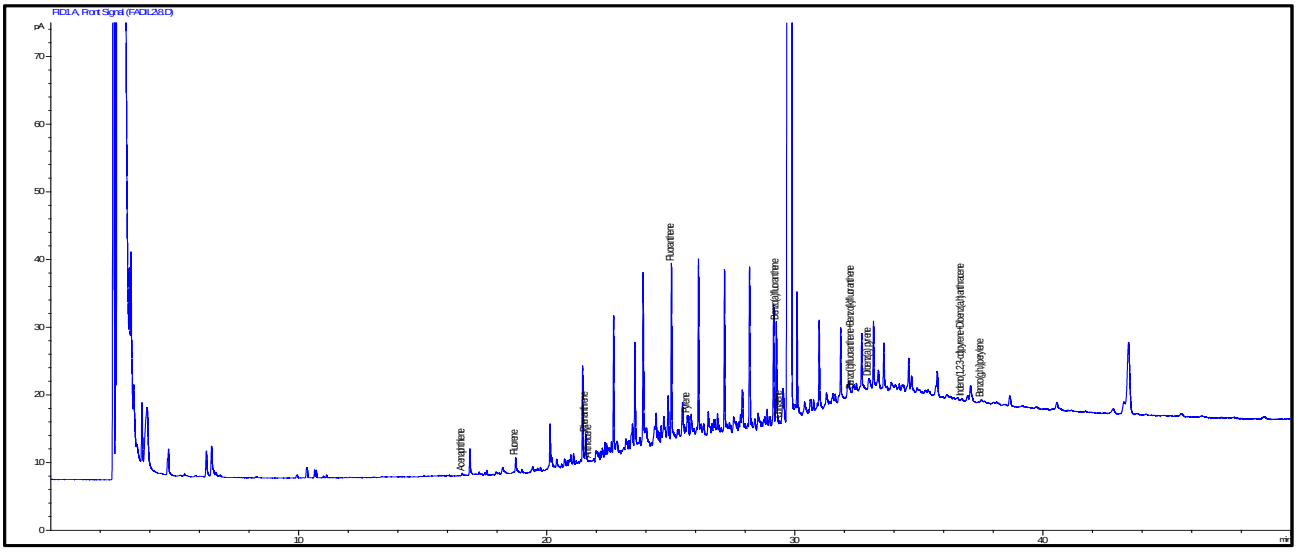

Figure 13 Crude oil 2.0% with *V. vulnificus*

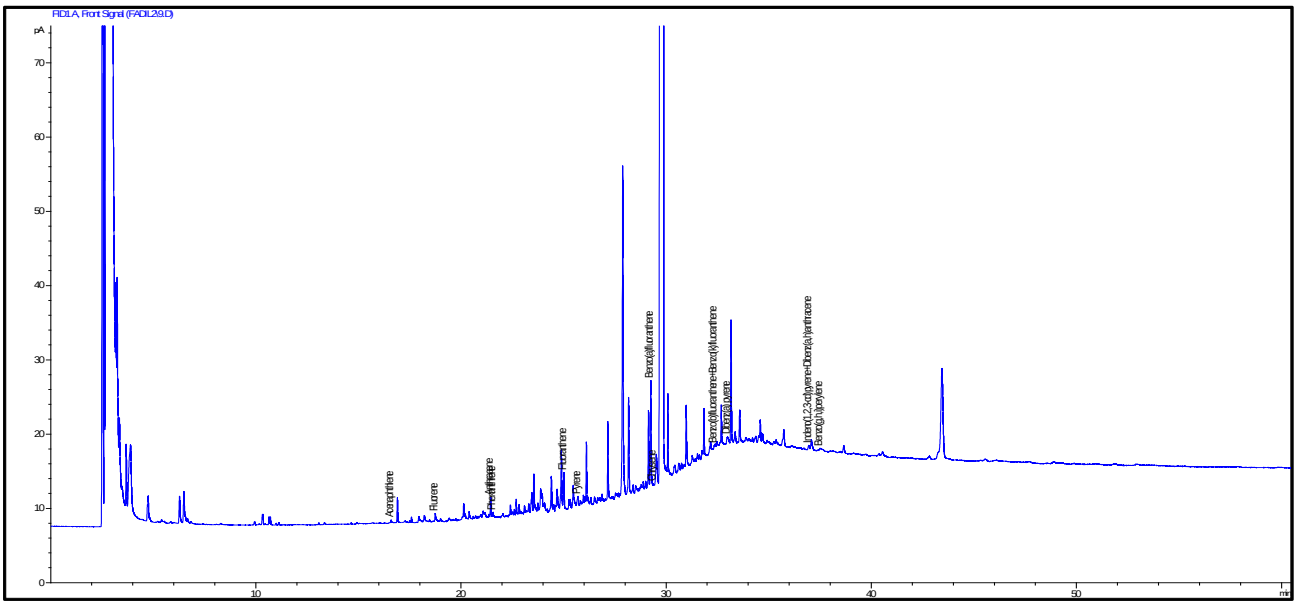

Figure 14 Crude oil 0.25% with *Sphingomon. Paucimobilis*

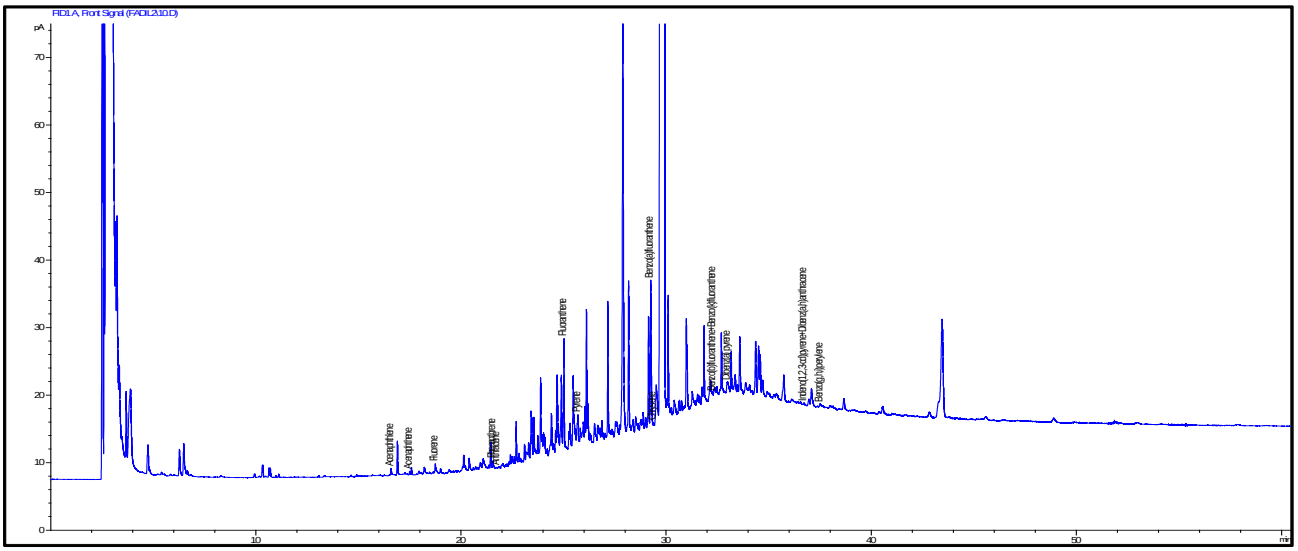

Figure 15 Crude oil 0.5% with *Sphingomon. paucimobilis*

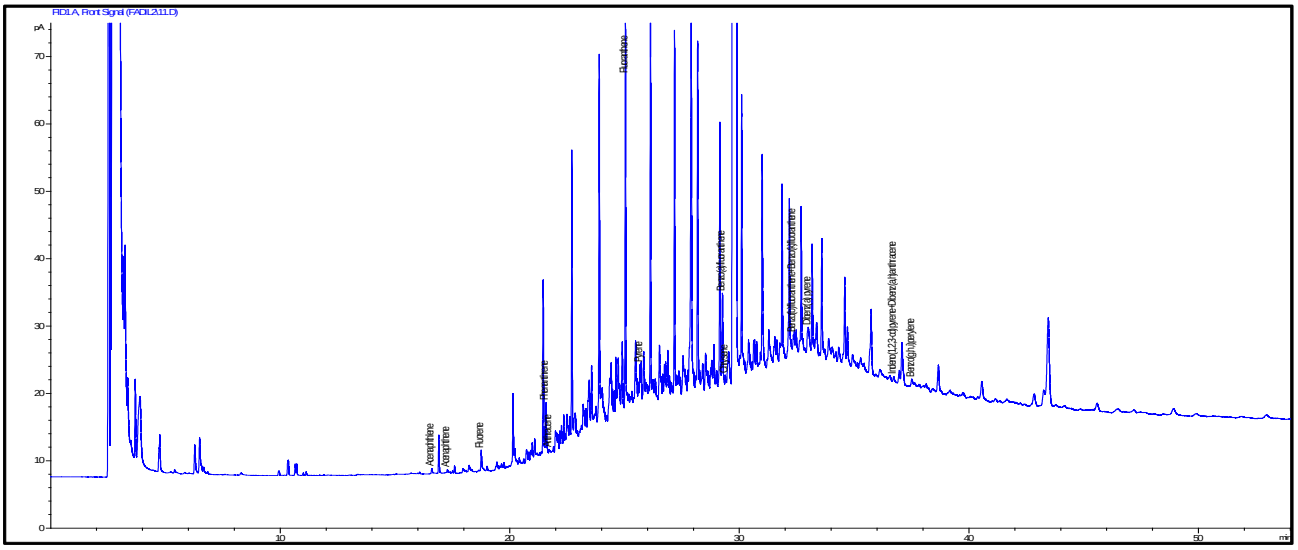

Figure 16 Crude oil 1.0% with *Sphingomon. paucimobilis*

Pure and mixed cultures of bacteria isolated from fresh water and seawater have the capacity to metabolize, anthracene, phenanthrene and as the only source of carbon anthracene are completely mineralized. *Pseudomonas*, *Sphingomonas*, *Nocardia*, *Beijerinckia*, *Rhodococcus* and *Mycobacterium* with the initial oxygenated intermediate being a dihydriol (Mrozik et al., 2003).

While *Ochrobactrum anthropic* showed low degradation percentage of all concentrations (Table 2; Figure 18; Figure 19; Figure 20; Figure 21). Sulaiman et al. (2016) found that, out of 35 isolates obtained from petroleum-contaminated soil only five isolates have the ability to degrade phenanthrene and anthracene. Whereas Katsivela et al. (2002) found that, 97% of 2; 2; 4; 4; 6; 8; 8- heptamethylnonane, 55% of toluene, 71% of acenaphthylene and 72% of acenaphthene were depleted after 9 days of growth. The ability of these bacteria to remove different PAHs looks promising for use in bioremediation technologies.

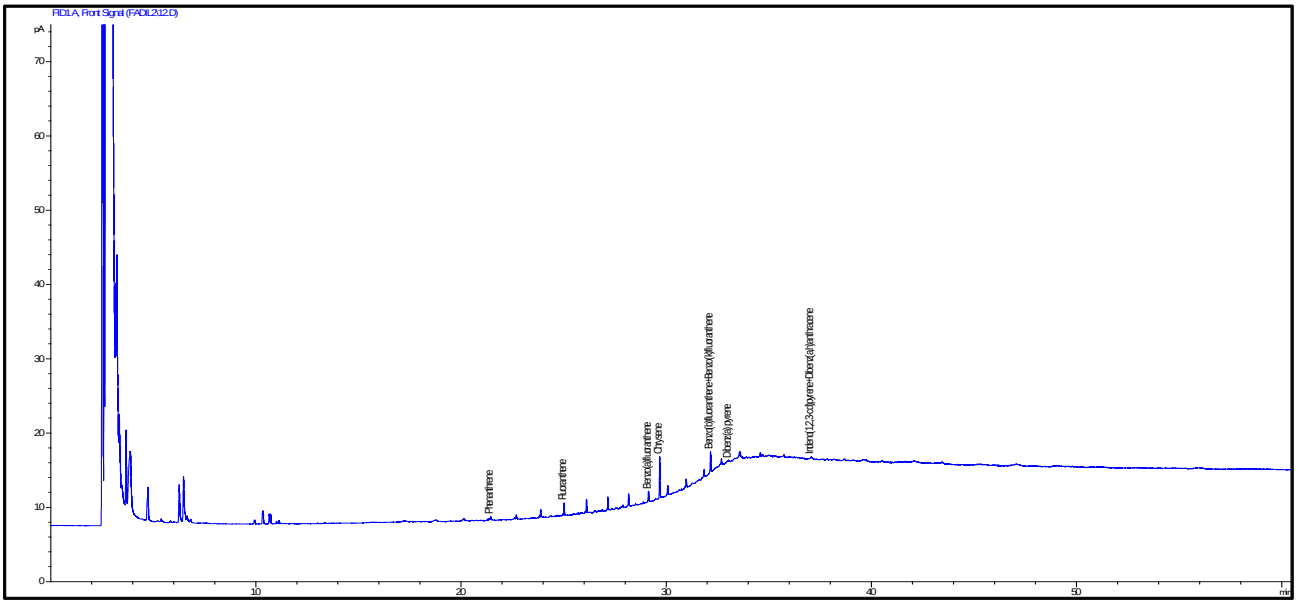

Figure 17 Crude oil 2.0% with *Sphingomon. paucimobilis*

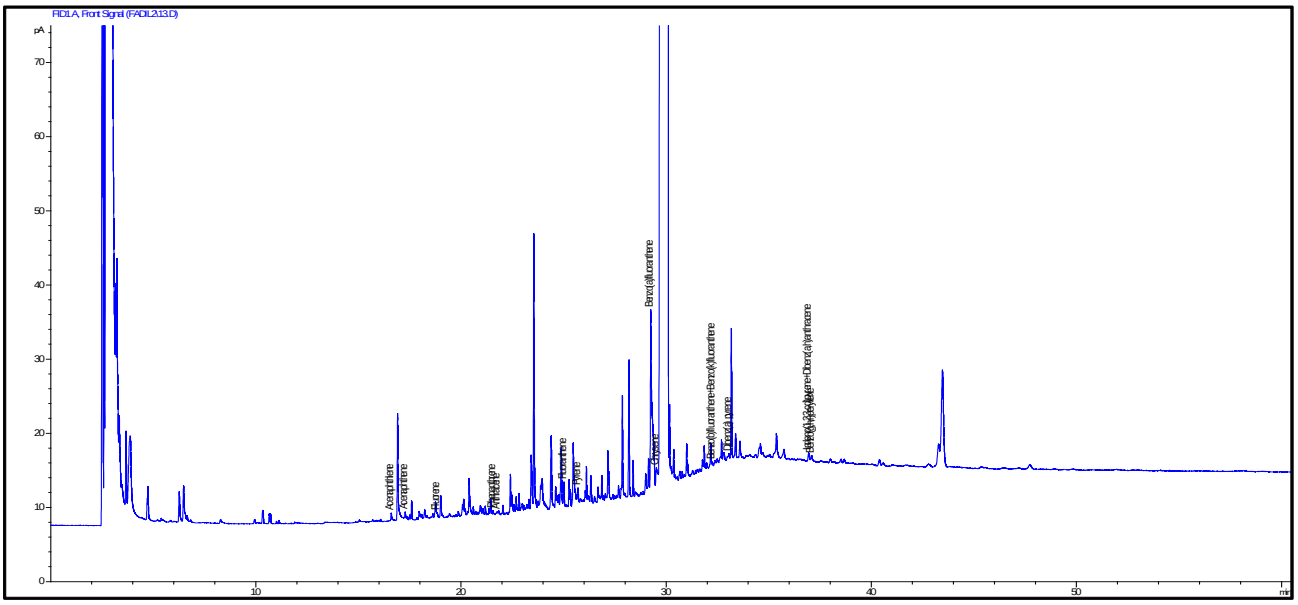

Figure 18 Crude oil 0.25% with *Ochrobac. anthropi*

Biodegradation of PAHs as described by Johnsen et al. (2005) can serve three different functions:

1- Assimilative biodegradation that yields carbon and energy for the microorganism and goes along with the mineralization of the compound.

2- Intracellular mechanism of PAHs is soluble in water and therefore causes excretion.

3- Cometabolism which is the degradation of PAHs without production of energy and carbon for the organism metabolism.

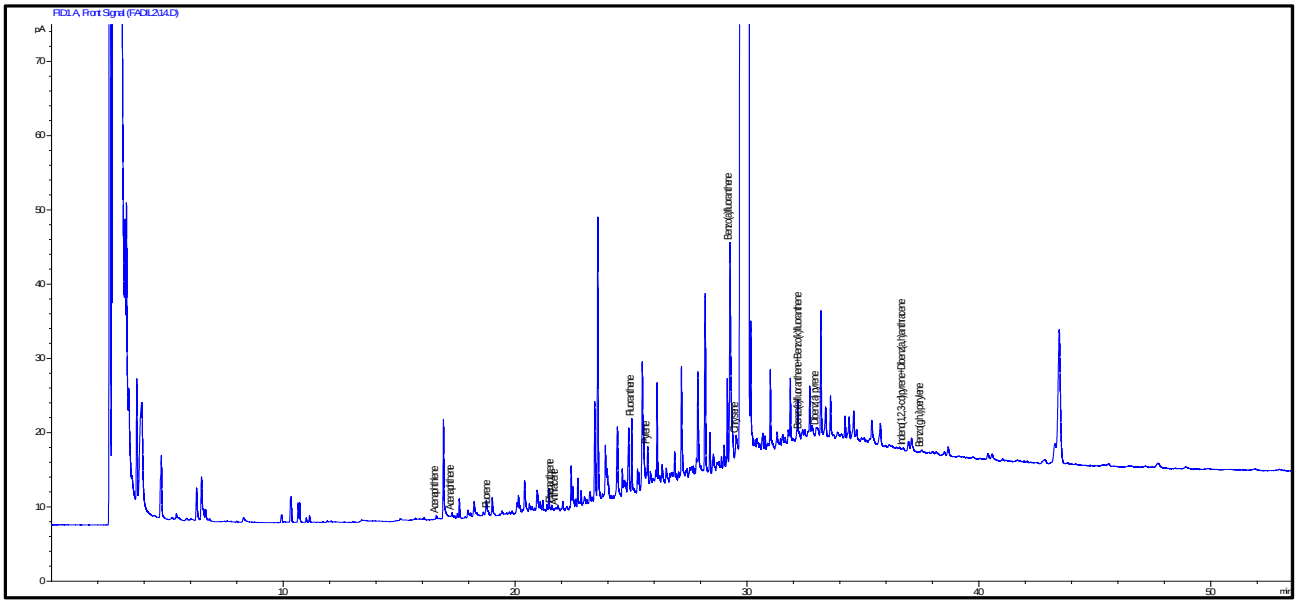

Figure 19 Crude oil 0.5% with *Ochrobac. anthropi*

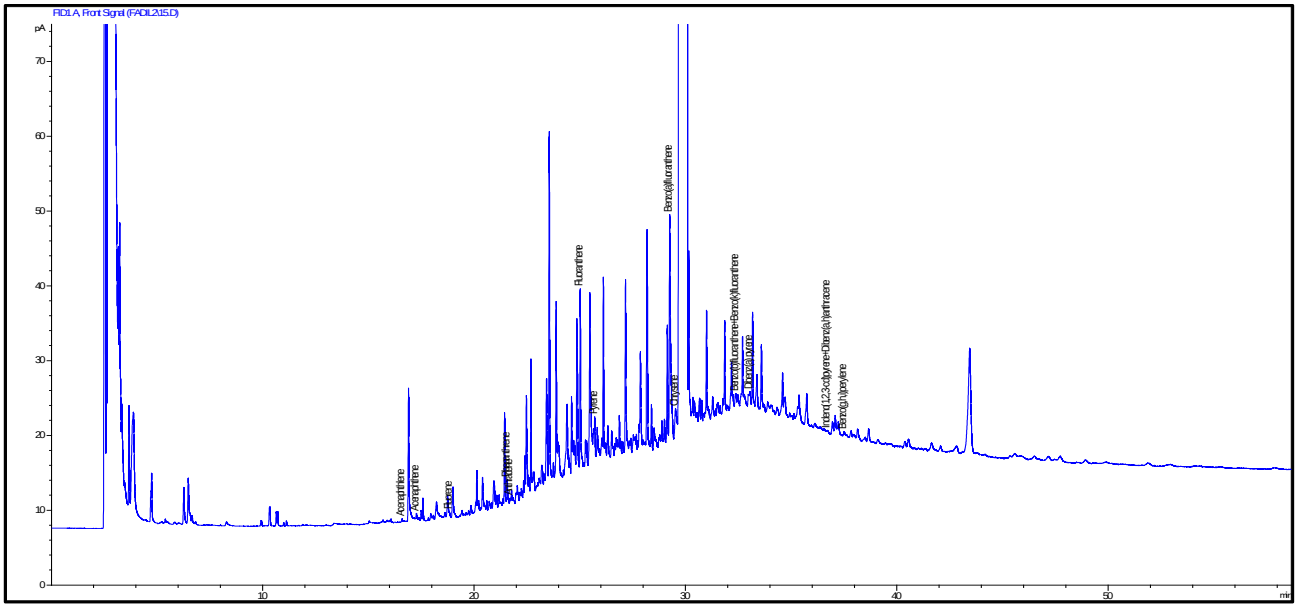

Figure 20 Crude oil 1.0% with *Ochrobac. anthropi*

These differences between the bacterial degradation of only the PAH environment probably reflect the diversity in the catabolic systems PAH and general physiology and possibly mediated ecological differences that are not understood (Brian et al., 2001).

The degradation of aliphatic hydrocarbons and PAH was mainly of the mono- and dioxygenase produced by bacteria. Therefore, the presence of six key enzymes coding genes, including both monooxygenase and

dioxygenase in the ASU-06 strain of *S. koreensis* the existence of monooxygenase genes (alkB and alkB1) dioxygenase (naháč) and Catechol dioxygenase (*C12O* and *C23O*) genes was confirmed. Therefore, one possible reason may be that these genes are strong evidence between different conserved Gram-negative bacteria (Hesham et al., 2014).

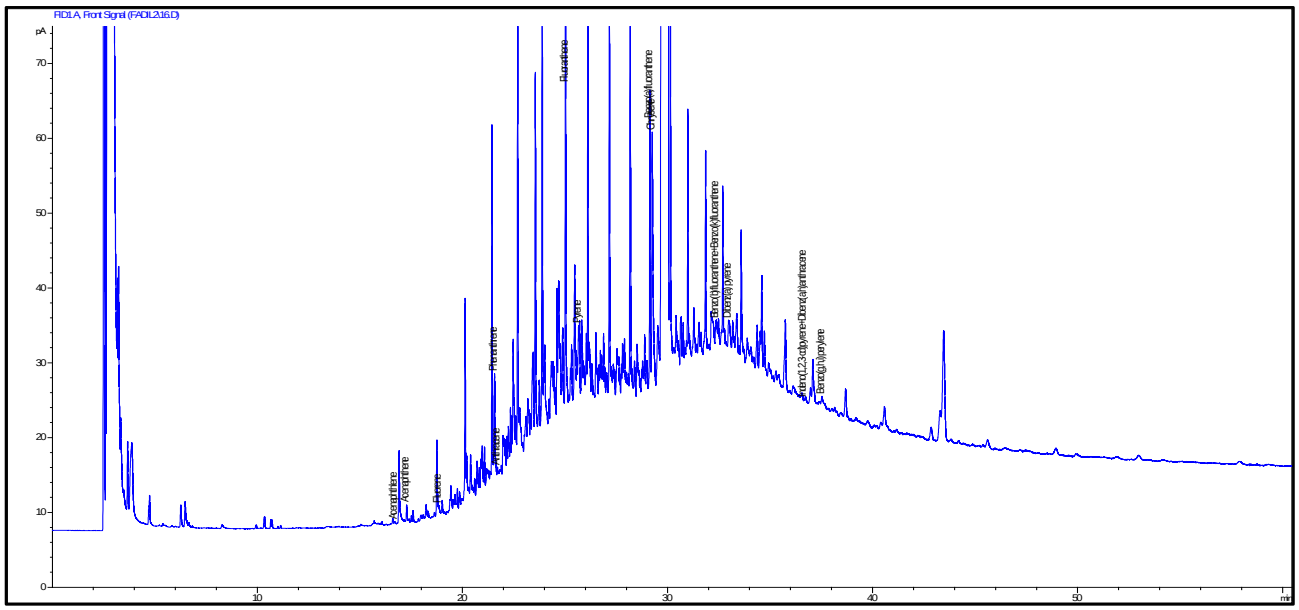

Figure 21 Crude oil 2.0% with *Ochrobac. anthropi*

3 Conclusion

In this study, 4 oil degrading bacterial strains were isolated from brackish water. The bacteria were identified as *Brevundimonas diminuta/ vesicularis*, *Vibrio vulnificus*, *Sphingomonas paucimobilis* and *Ochrobactrum anthropic* based on morphological properties and by Vitek II system. We found a variation in their capabilities to degrade PAH and most effective in higher concentrations. Perhaps, this is related to the types of bacteria and the mechanisms which to degrade crude oil.

Authors' contributions

AMR designed and conducted research, and also arranged all things to complete this paper. Co-authors are contributed to collection samples from Khor Al-Zubair channel, southern Iraq and analyze the samples.

Acknowledgments

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