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Microbial Diversity of Long-Duration Gas Injection Oil Reservoir Based on Next Generation Sequencing in South of Iran

Mohsen Pournia*(**), Nima Bahador† and Ghasem Hosseini Salekdeh*****

*Department of Microbiology, Science and Research Branch, Islamic Azad University, Fars, Iran

**Department of Microbiology, College of Science, Agriculture and Modern Technology, Shiraz Branch, Islamic Azad University, Shiraz, Iran

***Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

†Corresponding author: Nima Bahador

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ABSTRACT

Recent studies have shown that oilfield harbours diverse microbial communities. Water-flooding is believed to be the main contaminating factor of oil reservoirs, however, less attempts have been made to study the effect of natural gas injection on microbial community of oilfields. Molecular methods were used to evaluate the microbial diversity of Haftkel (HK) and Lali (LA) petroleum reservoirs in the south of Iran. The HK oilfield has been injected with natural gas for long duration, but LA oilfield had no enhanced oil recovery (EOR) process. Next generation sequencing (NGS) of 16S rRNA archaeal genes indicated that the genus *Methanofollis* was observed with high presence in 89.7% and 92.6% frequency in the HK and LA oilfields, respectively. Most abundant phyla in HK oilfield were Synergistes (58.1%), Firmicutes (17%), Proteobacteria (Betaproteobacteria) 12.8% and unclassified bacteria (5.2%), while Thermotogae (78%), Firmicutes (10.8%), Synergistes (4%) and unclassified bacteria (3.8%) were observed in LA oilfield. The comparison of the results indicated that the injection of natural gas could increase bacterial diversity and most probable cause to increase frequency of bacterial genus *Anaerobaculum* belonging to the Synergistes.

INTRODUCTION

There are large number of the petroleum reserves in world, which show the existence of a specific range of indigenous bacteria, with diverse physiological and metabolic functions and phylogenetic relationships. These bacteria have adapted to reservoir's conditions during several years (Youssef et al. 2009, Ollivierand Magot 2005, Magot et al., 2000). Contamination of oil reservoirs occurs during sampling and enhanced oil recovery (EOR) processes which affects the native microbial community (Youssef et al. 2009, Ollivierand Magot 2005, Fan Zhang et al. 2010). The water-flooding is the main contaminating factor of oil reservoirs; however, there is a challenge to understand the microbial community during gas injection (Youssef et al. 2009, Ollivierand Magot 2005, Magot et al. 2000, Fan Zhang et al. 2010).

Normally, oil reservoirs provide an appropriate environment for anaerobic and facultative anaerobic microorganisms (Ollivierand Magot 2005, Magot et al. 2000). Major electron donors are hydrogen, volatile fatty acids, petroleum hydrocarbons and inorganic electrons, while sulphate and carbonate minerals play important role as electron acceptors in many oil reservoirs. Therefore, the main metabolic processes of indigenous microbes in petroleum reservoirs are sulphate reduction, iron reduction, fermentation, acetogenesis and methanogenesis (Youssef et al. 2009, Ollivierand Magot 2005, Magot et al. 2000, Fan Zhang et al. 2010).

Culture dependent approaches are considered to reveal limited efficiency to describe the microbial communities of ecosystems completely (Martin & Karsten 2014), while culture-independent 16S rRNA gene based investigations and metagenomic study are extremely valuable in providing an overall view of the community composition in a specific ecosystem (Martin & Karsten 2014).

Hence, this is the first molecular study conducted to determine the microbial communities of crude oil samples obtained from a petroleum reservoir, which has been injected by natural gas. The results were then compared with another similar and close oilfield (LA) with no natural gas injection in south Iran and Middle East oilfields.

MATERIALS AND METHODS

Reservoir conditions: All samples originated from the Haftkel (HK) and Lali (LA) oilfields, located at the south of Iran. The distance between HK and LA oilfield is about 70 km. The production of the crude oil from HK oil field was started in 1927; this reservoir has a small gas cap and a fracture reservoir using the miscible gas injection, which could increase the oil recovery factor. Hydrocarbon con-

tents of both oilfields were almost similar, but no EOR processing has been performed in LA oil reservoir.

Sampling: Five litres of HK crude oil samples were collected from two distinct operating oil wells (HK-1 and HK-2) and five litres LA produced water obtained from its oil-water processing site (Haft-Shahid), during the period of June to October 2015. The bottles were filled completely and sealed to maintain anoxic conditions and immediately taken to the laboratory for extraction of DNA (Korenblum et al. 2012, Irene et al. 2008).

DNA extraction: Since the amount of water accompanied by the HK crude oil was low, 100 mL of each sample was mixed with the same volume of the sterile Winogradsky's buffer (Korenblum et al. 2012, Irene et al. 2008). The aqueous phase was separated from crude oil by decantation at room temperature. This procedure was repeated five times, until all of the crude oil was mixed and about five litres of aqueous phase were obtained. All aquatic phases and five litres of LA produced water were filtered directly using 0.2μm Startolab 150 V filter (Sartorius Biotech). All filters were placed in 5 mL homogenizer buffer (100 mM Tris-HCl (pH 8.2); 100 mM EDTA (pH 8); 1.5 M NaCl) and incubated under shaking conditions overnight for re-suspended microbial cells (Grabowski et al. 2005, Siddhapura et al. 2010). The genomic DNA extraction was performed in a harsh manner which combined several lysis methods together according to Siddhapura et al. (2010). The concentrations of DNA in the extracts were determined using the Quant-iT dsDNA assay kit and the qubit fluorometer (Invitrogen, USA).

16S rRNA gene amplicon library and high-throughput sequencing: The bacterial diversity was studied by pyrosequencing the amplified V1-V3 region of the 16S rRNA gene amplifying a fragment of 520 bp using the 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 534R: 5'-ATTACCGCGGCTGCTGG -3' primers (Tasharrofi et al. 2011). 454-adaptors were included in the forward primer, followed by a 10 bp sample-specific Multiplex Identifier (MID). PCR amplification was done using 1X enzyme buffer, 0.2 mM dNTPs mixture (Fermentas), 1U High-Fidelity DNA Polymerase (Fermentas), 0.5 μM forward and reverse primers, 1.5 mM $MgCl_2$ and 2 μL of the diluted DNA sample. The archaeal community analysis was carried out by pyrosequencing the amplified 457 bp region of the 16S rRNA gene amplifying using the 349F: 5'-GYGCASCAGK CGMGAAW-3' and 806R: 5'-GGACTACVSGGG TATCTAAT-3' primers with PCR reaction conditions as same as above (Takai et al. 2000). PCR incubation for bacterial and archaeal 16S amplicon libraries construction were performed according to the conditions described: 95°C for 2 min, followed by 30 cycles of 95°C (20s), 56°C (30s), 72°C (60s) with final extension 72°C (5 min) and 94°C for 3 min, followed by 35 cycles of 94°C (45s), 50°C (60s), 72°C (90s) with a final extension of 72°C for 10 min. After agarose gel electrophoresis, PCR products were purified and then quantified using the Qubit fluorometer. An equimolar pool was obtained prior to further processing. The amplicon pool was used for pyrosequencing on a GS junior platform (454 Life Sciences, Roche, Macrogen) according to the manufacturer's instructions using titanium chemistry.

Bioinformatics analysis: Raw reads were firstly filtered according to the 454 amplicon processing pipeline. Sequences were then analysed by QIIME 1.6.0 software (Caporaso et al. 2010). Raw reads were demultiplexed and further filtered through the split_library.py script of QIIME. For 16S rRNA, gene reads and the analysis was carried out as follows: Sequences that passed the quality filter were denoised and singletons were excluded. OTUs defined by a 97% of similarity were picked using the uclust method and the representative sequences, chosen as the most abundant in each cluster, were subjected to the RDPII classifier to obtain the taxonomy assignment and the relative abundance of each OTU using the Greengenes 16S rRNA gene database. Alpha and beta diversity were evaluated through QIIME as recently described. Connections were drawn between samples and OTUs, with edge weights defined as the number of sequences from each OTU that occurred in each sample. Network was visualized using Cytoscape 2.5.2 (Caporaso et al. 2010).

RESULTS AND DISCUSSION

Physicochemical characteristics of HK and LA oil fields: The characteristics of HK and LA oil reservoirs with typical hydrocarbon contents and properties of the natural gas cap according to internal report of National Iranian South Oil Company (NISOC 2014) are given in Table 1.

The injection of the natural gas into HK oil field studied has been made since 1976, typical with following characteristics per (mole %): 82% CH_4, 10% C_2H_6, 3.5% C_3H_8, 1.7% C_4H_{10}, 0.6% C_5H_{12}, 0.3% C_6H_{14}, 0.7% C_7H_{16}, 1.2% nitrogen, 0.2% carbon dioxide, hydrogen sulfide etc. in trace range.

Phylogenetic association of archaeal 16S rRNA gene sequences: The 16S rRNA sequencing of archaeal genes retrieved from HK samples indicated the abundance of 5 different archeal genera with one unclassified genus, which belonged to the phylum of Euryarchaeota. The genus *Methanofollis* was observed with the frequency of 89.65%, while genera of Methanothermobacter and Methanobrevi bacter were characterized with 3.25% and 1.2% respectively. Furthermore, genera *Methanomethylovorans* and *Haloarcula* were characterized with 0.7% frequency, ac-

Table 1: Physico-chemical characteristics of the HK and LA oil reservoirs.

Parameters	HK oil field	LA oil field
Temperature (°C)	45-47	56
pH	8.1	7
Depth (m)	800	1500
API gravity	34.5	37
Total sulfur in oil (w/w %)	0.92	0.70
ΣH_2S (ppm)	650	920
Salinity (ppm)	228.000	210.000
Chemical characteristics (ppm)[a]		
$K^+ + Na^+$	68520	48870
Mg^{2+}	7200	1944
Cl^-	14200	12900
SO_4^{2-}	1700	900
Ca^{2+}	10000	18800
HCO_3^-	1220	730
GC chromatogram of the HK oil sample[b]		
% Saturate	65/76	57
% Aromatic	29/14	29/7
% Resin	3.59	11/3
% Asphaltenes	1/15	1/4
Pristane/Phytane	1/18	1/05
C_{19}/C_{20}	1/25	1/1
C_{21}/C_{22}	1/21	1/37
C_{23}/C_{24}	1/3	1/4
Natural gas cap (mole %)		
CH_4	86	83.8
C_2H_6	4.7	4.8
C_3H_8	1.8	3.7
C_4H_{10}	0.9	1.2
C_5H_{12}	0.4	0.8
C_6H_{14}	0.3	0.4
C_7H_{16}	0.2	0.6
Nitrogen	0.6	0.1
CO_2	4.6	0.7
H_2S	0.6	3.96

a - Results of analysis HK oilfield formation water; b - Results of analysis HK # 34 well

companied by one unclassified genus of Halobacteriaceae with 4.5% predominance (Table 2). In addition, the frequency of archaeal community retrieved from LA oilfield included *Methanofollis* 92.6%, *Methanothermobacter* 3.2%, *Methanobrevibacter* 0.3% and *Methanother mococcus* 0.2% with one unclassified genus of Halobacteriaceae 0.1% (Table 2).

The *Methanofollis* is a mesophilic non-motive, obligately anaerobic, cell wall similar Gram negative, which consumes H_2/CO_2, 2-butanol/CO_2, and formate for methanogenesis. Some species of *Methanofollis* have been reported in Dagang oilfield (China), Saudi Aramco crude oil pipeline, onshore Potiguar basin (Brazil) and oil-gas petroleum reservoir in Higashi (Japan), with the relative high frequency (Silva et al. 2013, Pavlova et al. 2014, Shimizu et al. 2010, AlAbbas 2013). As given in Table 2, other methanogens were identified in a low frequency. *Methanothermobacter*, *Methanobrevibacter* and *Methanothermococcus* with hydrogenotrophic metabolism and *Methanomethylovorans* can use acetate, methanol, dimethyl sulphides, and methylamines has been identified from the other oilfields with the high temperature, such as Qinghuang (China), oil-water processing site (Netherlands) and Athabasca oil sands (Pavlova et al. 2014, AlAbbas 2013, Kraan et al. 2010, Li et al. 2007, Harner et al. 2011).

Rest of archaea identified belonged to the one unclassified genus *Haloarcula* of Halobacteriaceae. To the best of authors' knowledge this is the first report on the detection of *Haloarcula* in the petroleum reservoirs. Most members of this genus are heterotroph and obligate aerobes but some Haloarchaea grow anaerobically to reduce nitrate or fumarate (Fathepure 2014).

The diversity of the archaeal and bacterial communities for all samples was estimated using the Rarefaction curve and Shannon index (Fig. 1). The rarefaction curves suggested that the sequence population was less diverse for

Table 2: Total diversity of microbial community HK and LA oilfields.

Microorganism	HK (% Coverage)	LA (% Coverage)	Classification
Bacteria			
Bacteria; Other[a]	5.2 (4.6)	3.8 (3.4)	Bacteria
Cyanobacteria; Other	0.3 (0.2)	0.8 (0.71)	Cyanobacteria
Firmicutes; Other	NA [b]	0.2 (0.17)	Firmicutes
Proteobacteria; Other	0.2 (0.18)	NA	Proteobacteria
Betaproteobacteria; Other	2 (1.8)	NA	Betaproteobacteria
Propionibacteriaceae; *Propionibacterium*	3.8 (3.4)	1.6 (1.43)	Actinobacteria
Porphyromonadaceae; Other	0.4 (0.3)	NA	Bacteroidetes
Bacilli; Other	0.1 (0.09	0.3 (0.26)	Firmicutes
Bacillaceae; Other	0.5 (0.4)	NA	Firmicutes
Bacillaceae; *Bacillus*	7.5 (6.6)	5.9 (5.3)	Firmicutes
Bacillaceae; *Geobacillus*	NA	0.3 (0.26)	Firmicutes
Lactobacillales; Other	0.4 (0.3)	NA	Firmicutes
Lactobacillaceae; Other	0.3 (0.2)	0.2 (0.17)	Firmicutes
Streptococcaceae; Other	1.5 (1.3)	NA	Firmicutes
Streptococcaceae; *Lactococcus*	3 (2.6)	0.8 (0.71)	Firmicutes
Streptococcaceae; *Streptococcus*	0.3 (0.2)	0.3 (0.26)	Firmicutes
Staphylococcaceae ; *Staphylococcus*	0.4 (0.3)	1 (0.9)	Firmicutes
Streptococcaceae; Other	NA	0.6 (0.54)	Firmicutes
Leuconostocaceae; *Leuconostoc*	0.2 (0.18)	NA	Firmicutes
Clostridiales; Other	0.8 (0.7)	NA	Firmicutes
Clostridiaceae; Other	0.2 (0.18)	NA	Firmicutes
Clostridiaceae; *Thermoanaerobacterium*	0.8 (0.7)	0.2 (0.17)	Firmicutes
Clostridiaceae; *Fusibacter*	NA	1 (0.9)	Firmicutes
Peptococcaceae; *Sporotomaculum*	1(0.9)	NA	Firmicutes
Comamonadaceae; *Pelomonas*	0.5 (0.4)	NA	Betaproteobacteria
Rhodocyclaceae; Other	11.4 (10)	NA	Betaproteobacteria
Synergistaceae; *Anaerobaculum*	58.1 (51)	NA	Synergistes
Burkholderiales; Other	0.2 (0.18)	NA	Betaproteobacteria
Oxalobacteraceae; Other	0.2 (0.18)	NA	Betaproteobacteria
Oxalobacteraceae; *Ralstonia*	0.5 (0.4)	NA	Betaproteobacteria
Yaniellaceae; Other	0.2 (0.18)	NA	Actinobacteria
Thermotogaceae; *Petrotoga*	NA	78 (69.8)	Thermotogae
Synergistetes; *Thermovirga*	NA	4 (3.6)	Synergistes
Bradyrhizobiaceae; *Bradyrhizobium*	NA	0.2 (0.17)	Alphaproteobacteria
Sphingomonadaceae; Other	NA	0.4 (0.35)	Alphaproteobacteria
Caulobacteraceae; *Phenylobacterium*	NA	0.2 (0.17)	Alphaproteobacteria
Corynebacteriaceae; *Corynebacterium*	NA	0.2 (0.17)	Actinobacteria
Archaea			
Methanomicrobiaceae; *Methanofollis*	89.7 (11.2)	96.2 (10.1)	Euryarchaeota
Methanobacteriaceae; *Methanothermobacter*	3.2 (0.4)	3.2 (0.4)	Euryarchaeota
Methanobacteriaceae; *Methanobrevibacter*	1.2 (0.2)	0.3 (0.03)	Euryarchaeota
Methanosarcinaceae; *Methanomethylovorans*	0.7 (0.1)	NA	Euryarchaeota
Methanococcaceae; *Methanothermococcus*	NA	0.2 (0.02)	Euryarchaeota
Halobacteriaceae; other [a]	4.5 (0.6)	NA	Euryarchaeota
Halobacteriaceae; *Haloarcula*	0.7 (0.1)	NA	Euryarchaeota
Euryarchaeota; other	NA	0.1 (0.01)	Euryarchaeota

[a]Unclassified; [b]Not available

archaeal community in both the samples. The crude oil samples had different Shannon indices, indicating that these samples were distinct. The archaeal Shannon indices of two HK samples were higher than LA crude oil sample, suggesting that the archaeal diversity was more in the HK oil field, but in the LA reservoir, bacterial diversity was more (Fig. 1).

Phylogenetic association of bacterial 16S rRNA gene sequences: The sequence of 16S rRNA bacterial genes retrieved from HK oilfield included members of Synergistes 58.1%, Firmicutes 17%, Proteobacteria (Betaproteo bacteria) 12.8%, unclassified bacteria 5.2%, Actinobacteria 4%, unclassified Betaproteobacteria 2%, Bacteroidetes 0.4%, Cyanobacteria 0.3% and unclassified Proteobacteria 0.2% (Fig. 2).

The major group of bacteria belonged to the genus *Anaerobaculum* with the frequency of 58.1% (Table 2). These bacteria have been suggested to be classified as the

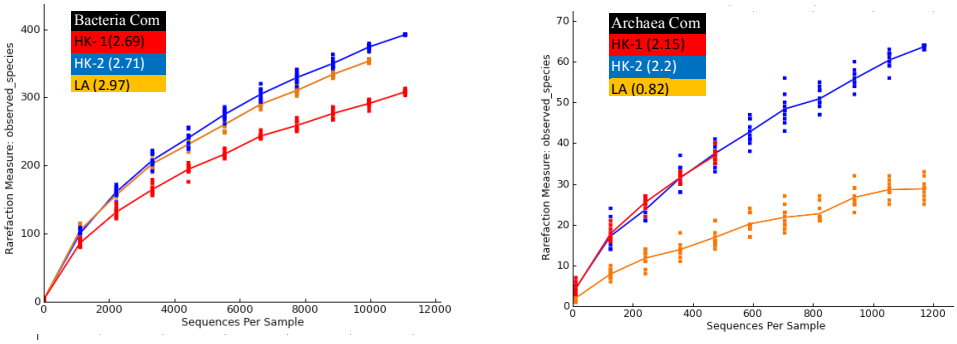

Fig. 1: The rarefaction curves represent the number of OTUs vs sequences per sample, archaea and bacteria. Numbers in parentheses are the Shannon indices average of each sample.

new phylum of Synergistes, family of Synergistaceae. The *Anaerobaculum* is Gram-negative, curved rod, motile, chemoorganotrophic anaerobe, non-spores forming and moderately thermophilic (35-65°C) with a growth pH range of 5.4-8.7. It can ferment a limited range of carbohydrates, organic acids, protein and glycerol to H_2, acetate and CO_2. This genus also reduces thiosulphate, cysteine and sulphur to hydrogen sulfide as electron acceptor. It has been found that *Anaerobaculum* as thiosulphate-reducing bacteria has a corrosive effect on some petroleum reservoir equipment (Rees et al. 1997, Liang et al. 2014). Among members of this phylum, mostly the genera *Anaerobaculum*, *Thermovirga* and *Dethiosulfovibrio* have been isolated from oil reservoirs (Pavlova et al. 2014, Kraan et al. 2010, Rees et al. 1997, Halim et al. 2015, Wang et al. 2012, Piceno et al. 2014, Alexandre 2015, Maune et al. 2012).

The other main groups included the family Rhodocyclaceae and genus *Bacillus* with the frequency of 11.4% and 7.5%, respectively (Table 2). Rhodocyclaceae has also very versatile metabolic capabilities (Das et al. 2014, Kadnikov et al. 2013). Among their members, *Thauera*, *Azoarcus* and *Dechloromonas* are known to be capable of degrading a large number of petroleum hydrocarbons under aerobic and anaerobic conditions in petroleum associated environments, sludge and reservoirs. Furthermore, their members use different substances like perchlorate, nitrate, ferric iron and other metals as electron acceptors during biodegradation of aromatic hydrocarbons. Some genera of this family like *Thauera* as heterotrophic nitrate-reducing bacteria (hNRB) were also found in oil reservoirs injected with nitrate and compete with SRB for degradable organic electron donors, and can prevent souring in the oil cavity environments (Fan Zhang et al. 2010, Irene et al. 2008, Das et al. 2014, Kadnikov et al. 2013, Hubertand Voordouw 2007).

A variety of genera related to the family of Bacillaceae, especially the genera *Bacillus* and *Geobacillus* have been identified from different oil fields, oily sludge, sand oils and oil pipelines. It seems that the existence of the spore in these genera help them to survive in harsh conditions and penetrate deep into petroleum reservoirs. Potential of production of some acids, gas, alcohols and biosurfactants make them the ideal bacteria to be used in developing the MEOR technology and bioremediation of oil pollution (Youssef et al. 2009, Korenblum et al. 2012, Li et al. 2007, Harner et al. 2011, Das et al. 2014, Joshi et al. 2014, Lin et al. 2014).

Another sequence is most closely related to *Propionibacterium,* with 3.8% predominance (Table 2). Yoshida et al. suggested that these bacteria are indigenously stored crude oil with a lipolytic activity or hydrocarbon oxidation (Sette et al. 2007, Yoshida et al. 2005).

The orders Lactobacillales, Clostridiales and Thermoanaerobacterales were also detected in the HK oil reservoir, in which the dominant genera were *Lactococcus*, an unclassified genus of the family Streptococcaceae, *Sporotomaculum* and *Thermoanaerobacterium* (Table 2). Some species of *Lactococcus* were able to detect from different oil wells, oil wastewater and marsh sediments. This genus also produces biosurfactants and has been used in the oil recovery, drilling, bioremediation and removal of the heavy metal contaminants (Silva et al. 2013, Alexandre 2015, Machado et al. 2013, Gao et al. 2015, De Vos et al. 2009). The *Sporotomaculum* is rod shaped, Gram positive, strictly anaerobic, spore forming and fermentative but inorganic electron acceptors (sulphate, sulphite, thiosulphate, nitrate and ferric iron) are not used. Generally, this bacterium has been found in freshwater sediment, sludge and soil, which could metabolize benzoate, crotonate and butyrate in syntrophic association with hydrogenotrophic methanogen (Qiu et al. 2003). To the best of authors' knowledge, this is the first report on the detection of *Sporotomaculum* in this geographical area. The *Thermoanaerobacterium* sp. is extreme thermophile (35-75 °C), endospores in some species, obligate anaerobe, chemo-organotrophs with a variable ability to ferment carbohydrates and could reduce thiosulphate to elemental sulphur and might increase the risk of corrosion in the oil pipelines (De Vos et al. 2009, Singh et al.

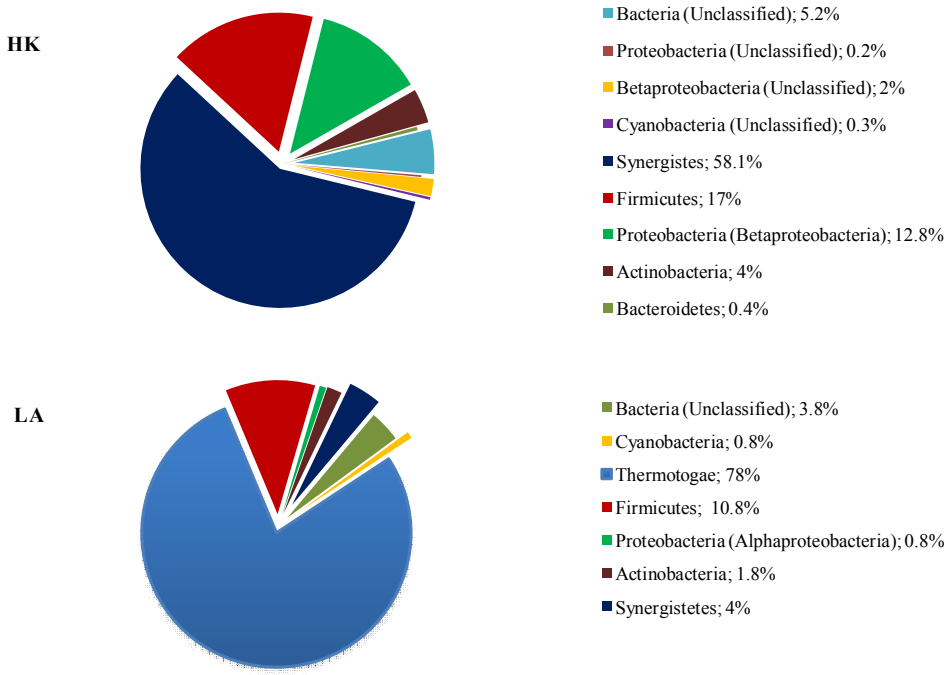

Fig. 2: Phylogenetic groups of bacteria detected within the HK and LA oil fields.

2014). The other bacteria were distinguished in the HK oilfield, with relative low abundances (less than 1% frequency) as given in Table 2.

In the LA crude oil, the main bacterial group belonged to the family of Thermotogaceae, and genus *Petrotoga* with the frequency of 78 % (Fig. 2). Other bacterial community with the relative abundance included the family Bacillaceae (*Bacillus* and *Geobacillus*) and Synergistaceae (*Thermovirga*) with the frequency of 6.5% and 4%, respectively (Table 2). Furthermore, other bacterial genera such as *Thermoanaerobacterium*, *Phenylobacterium*, *Bradyrhizobium*, *Corynebacterium*, unclassified genera of family Sphingomonadaceae and Lactobacillaceae were detected in the LA oilfield with less than 1% as given in Table 2.

Comparison of microbial community the HK and LA oilfields: Comparison of the results indicated that a lot of similarities between communities of archaea exist in both the oilfields and the most abundant genus was *Methanofollis*. As shown in Fig. 3, the bacterial diversity in HK oil field was more than LA, and the families Synergistaceae (*Anaero baculum*), Rhodocyclaceae and Bacillaceae were major groups of bacteria, while in LA oilfield, main bacterial families were Thermotogaceae (*Petrotoga*), Bacillaceae and Synergistaceae (*Thermovirga*).

Comparison of detected microbial community with Middle East oilfields: The comparison of HK oilfield with other oil reservoirs in Middle East such as Qatar and Arabian crude oil (Yamane et al. 2008), Bahja oil field in Omani (Elshafie et al. 2013) and Saudi Aramco oil sludge (Albokari et al. 2015) indicated that none of them showed the genus *Anaerobaculum*. However, AlAbbas et al. (2013) identified about 26% the family Synergistaceae in sour Arabian crude oil. Molecular study of these oilfields revealed that *Bacillus*, *Thermotoga*, *Petrotoga*, *Propionibacterium* and *Clostridium* were the most abundant genera. It is noteworthy that in these reservoirs a low diversity of archaeal community was detected, while in the oilfields tested the diversity of archaeal community were found in a higher amount. The study of other oil reservoirs in worldwide revealed that the *Anaerobaculum* was mostly detected in small amounts, but Halim et al. (2015), Pavlova et al. (2014) and Wang et al. (2012) showed that if crude oil was enriched with nutritive components, this bacterium increased greatly. Liang et al. (2014) also observed that co-culture of some methanogens with *Anaerobaculum* increased biofilm formation and biocorrosion of the metals in a medium with both yeast extract and elemental iron.

CONCLUSIONS

The present work was conducted for the first time in the Iranian oilfields arranged by molecular techniques. The archaeal communities in these oilfields were dominated by 6 diverse genera of Euryarchaeota phylum. The genus *Methanofollis* observed with high frequency and other methanogens such as *Methanobrevibacter*, *Methanother*

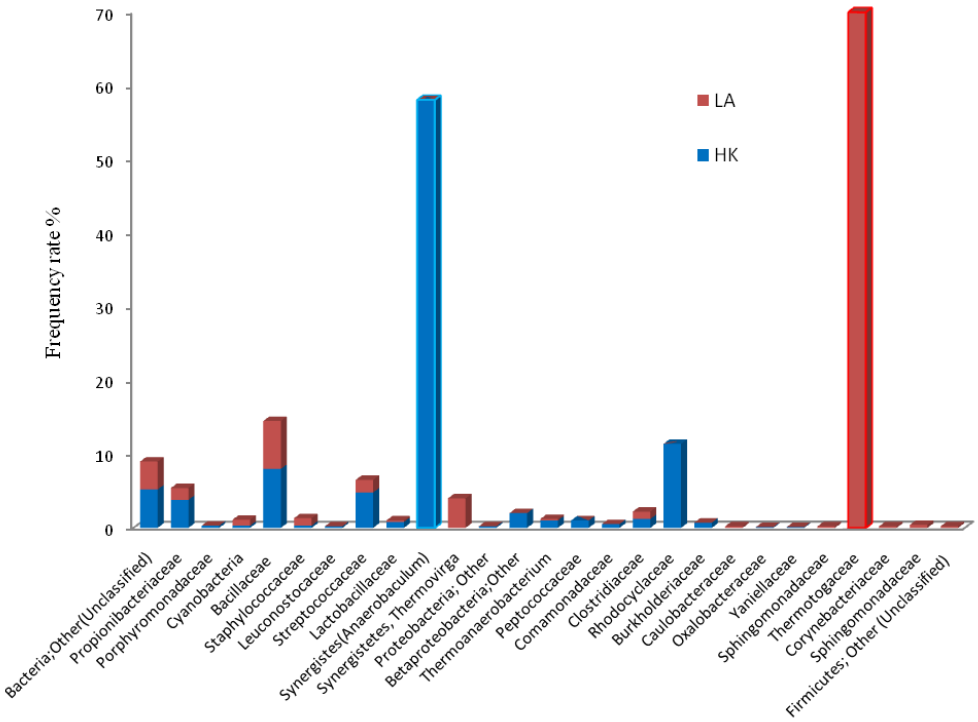

Fig. 3: Comparison of bacterial community of HK and LA oil fields.

mobacter, *Methanomethylovorans* and *Methanother mococcus* were also distinguished in the HK and LA reservoirs, with a low abundance. It seems that these methanogens are not so metabolically active or the growth conditions were not suitable for them. Moreover, based on these results, despite the low presence of Haloarchaea in oilfields, one unclassified genus of Halobacteriaceae 4.5% *Haloarcula* was detected with 0.7% frequency.

According to the 16S rRNA bacterial genes, most of the phyla obtained from HK oilfield were related to Synergistes, Firmicutes and Proteobacteria (Betaproteobacteria). The *Anaerobaculum* (58.1%) and one unclassified genus of family Rhodocyclaceae (11.4%) were observed more than the others. Probably it could be concluded that, injection of miscible flooding natural gas and dissolving the light hydrocarbon compounds of gas in oil phase, which can enrich the crude oil and may provide suitable conditions for growth of the genus of *Anaerobaculum*. In the LA crude oil, the main bacterial group belonged to the families Thermotogaceae (*Petrotoga*), Bacillaceae (*Bacillus* and *Geobacillus*) and Synergistaceae (*Thermovirga*) with the frequency of 78 %, 6.2% and 4%, respectively.

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