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Response of Microbial Community Structure to Different Enhanced Oil Recovery Processes in Shengli Oilfield (China)

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Aim to study that different enhanced oil recovery processes influence microbial community in the oil reservoir, three different types of samples from Ng3 zone of Shengli oilfield were analyzed: produced water from water-flooded well, from heterogeneous combination flooding well and from microbial enhanced oil recovery process. Based on 454 pyrosequencing of 16S rRNA gene amplicons, microbes clustered into Proteobacteria were dominantly detected in the three samples. However, Dominant microorganisms in three production wells were significantly different at genus level. The majority of reads obtained in three samples were aligned closely to *Azospirillum*, *Pseudomonas*, and *Thauera*, respectively. The microorganisms usually detected in oil reservoirs environments and associated with members of *Azospirillum*, *Pseudomonas*, *Thauera*, *Acinetobacter* and *Petrobacter*. Microbial communities in production well after nutrients injection, revealed an activation of the microbes, and some microorganisms were significantly inhibited with a sharp decline in the number. This first investigation of the microbial diversity in oil reservoir with different enhanced oil recovery processes expands substantially the knowledge of the extent of microbial diversity and highlights the complexity of microbial communities.

Key words: 454 pyrosequencing, microbial community, enhanced oil recovery (EOR).

It is a well-established fact that oil reservoirs harbor diverse microbial communities. Natural untouched oil reservoirs usually have low redox potentials and limited electron donors and acceptors, only strict anaerobes could normally survive, be active and truly considered indigenous[1]. The microbial ecology of oil reservoirs are usually disturbed and significantly altered during the production process and activities such as drilling, oil extraction and oil recovery processes[2]. These activities could invariably result in the introduction of some exogenous microorganisms which may survive and disappearance of some indigenous communities. It is not therefore surprising to detect a majority of facultative and aerobic microbes in samples from oil reservoirs under production[3-7]. On this regards, water-injection is believed to be the main factor involved in introducing and influencing the occurrence of new microbial communities of water-flooded oil reservoirs[2]. In a recent investigation of microbial ecology of water-flooded oil reservoir concluded that microorganisms present in the injected water has major effects on the microbial compositions of oil reservoirs[7]. Additionally, microbial enhanced oil recovery (MEOR)

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processes have been proved to be an effective tertiary oil recovery[8-11]. And nutrition injection would also result in changes of microbial diversity and community in oil reservoirs. Microbial community effected by different enhanced oil recovery (EOR) processes was not still reported. The investigation of microbial populations in oil reservoirs is a significant strategy to understand microorganisms that could inhabit oil reservoirs. Knowledge of microorganisms inhabiting oil reservoirs is also important for understanding the microbial ecology of oil reservoirs and the efficient applications involving augmentation of indigenous microbes or utilization of endogenous ones in oil production applications[2]. Molecular biological techniques have been reported to monitor changes in microbial communities during MEOR field trials[11-13]. Such sequencing data of traditional clone libraries could be an underestimation because detected representative operational taxonomic units (OTUs) were not sufficient to cover microbial diversity. And thus, in-depth knowledge of microbial communities could be attempted through the use of high-throughput sequencing technique. 454 pyrosequencing of 16S rRNA gene amplicons was used as powerful tool to detected microbial community in the production wells[14].Therefore, this technique was introduced tostudy microbes inhabiting Shengli oilfield reservoir (China).

In the study, the two main objectives were: (1) to compare the microbial communities in three production wells with different enhanced oil recovery processes to understand the relationships between these microbes and the oil reservoir, (2) to analyze microbial community after MEOR process in the oil reservoir to identify the stimulated microbial groups that could contribute be to enhance oil recovery.

MATERIALS AND METHODS

Sample collection

Ng3 zone is located in Shengli Oilfield (China). The oil reservoir is at approximately a depth of 1190 m, and has an *in situ* temperature of approximately 65°C. The viscosity of underground crude oil is 46.3mPa.s, and the formation water salinity is about 3850mg/l. Water-flooding process was carried out in Ng3 zonesince 1974. After the implement of polymer flooding process in the Ng3 zone since 1992, water-flooding process was implemented in production well 5N101; MEOR process was carried out in production well 7XNB11; the process of heterogeneous combination flooding was applied to production well 11XN411. Oil-water liquids from the wellheads of 5N101 (N5), 7XNB11 (X7) and 11XN411 (X11) were collected from the wellheads in sterile plastic bottles on October 17, 2012, and then transported to the laboratory as soon as possible for further analysis.

DNA extraction

Approximate 200 ml of oil-water samples were centrifuged to pellet cells. Following the manufacturer's instruction for TIAN Micro DNA Kit (TIANGEN Biotech Co., Ltd., China), genomic DNA was extracted in triplicate and mixed together. DNA were purified with an Agarose Gel DNA Purification Kit (TianGen Biotech, Beijing, China) and stored at -20 ^{o}C.

PCR amplification, amplicon quantitation, pooling and pyrosequencing

To identify microorganisms in each sample, 454 pyrosequencing of 16S rRNA gene were carried out. A region approximate 526 bp covering V1–V3 region of 16S rRNA gene in each sample was amplified using the primers 27F and 533R with the A and B adaptors (454 Life Science). PCR was carried out using template DNA following the previous PCR-reacting system and thermal cycle programme[15]. After amplification, replicate PCR products of the same sample were pooled and purified using DNA gel extraction kit (Axygen, China).

In preparation for sequencing, the concentration of each PCR product was measured using a Quant-iT PicoGreen double-stranded DNA assay (Invitrogen, Germany), and the quality of each PCR product was controlled on an Algilent 2100 bioanalyzer (Agilent, USA). Working pool was a mixture of equimolar rations of each amplicons and subjected to emulsion PCR to generate amplicon libraries, as recommended by 454 Life Science. Amplicon pyrosequencing were performed from the A-end using 454/Roche A sequencing primer kit on a Roche Genome Sequencer GS FLX Titanium platform (Majorbio Bio-Pharm Technology Co., Ltd, Shanghai, China).

Statistical and bioinformatics analysis

The raw multiplexed sequence reads were processed according to QIIME-1.6.0 Pipeline. After

a mapping file was generated, the multiplexed reads were assigned to samples based on their unique nucleotide barcodes in the step of split_libraries.py. During this step quality filtering to remove any low quality or ambiguous reads was carried out. Then, the pick_otus_through_otu_table.py workflow was run to assign sequences to OTUs at 97% similarity by default, to pick representative sequences for each OTU, to assign taxonomic data to each representative sequence based on RDP classifier by default, to align OTU sequences using the Greengenes file by default and to get OTU table. The obtained BIOM file formatted OTU table was converted to tab-delimited table including the taxonomy metadata by running conert_biom.py workflow.

Excel serviced as a statistic tool using the programs of AutoFilter and PovitTable. Notably, in this step, the data related with OTUs with sequences failed in alignment and unclassified to be bacteria were removed. Shared OTUs analysis was calculated with the Venn Diagram package in R (version 2.15.3). Rarefaction curves were made with the software of Analytic Rarefaction, and then thenumber of OTU vs. the number of reads in each library was calculated. Taxonomic richness and diversity analysis were also carried out as described by Caporaso *et al.*[16]. In this study, OTUs defined by ≥10 reads in any sample are "dominant OTUs". Sequences of dominant OTUs were used to identify microorganisms.

RESULTS AND DISCUSSION

Estimation of richness and diversity of microbes

A total of 26,307 valid reads from the three clone libraries (N5, X7 and X11)were obtained, and clustered into 1,003 OTUs through 454 pyrosequencing and bioinformatic analysis. The entire set of the raw reads was available at NCBI Sequence Read Archive (SRA) under accession number of PRJEB4792. The number of sequence reads was 7974, 6848, and 6889 for N5, X7 and X11, respectively, with OTUs ranging from 185 to 424(Tab.1). According to the reproducibility thresholds determined, 154 OTUs were grouped as dominant OTUs. Notably, although the number of OTUs decreased sharply, the number of reads clustered into these dominant OTUs decreased slightly in each sample. Valid reads that have similarity above 97% were clustered into OTUs to calculate the rarefaction and to analyze the taxonomic richness and diversity. Rarefaction curves of the bacteria diversity showed that three curves became flat at high values of number of sequence, indicating a good coverage of the species in the three production wells (Fig. 1).

Table 1. Evaluation of original and dominant OTUs and reads in the samples (N5, X7, and X11) based on 97% similarity clustering

Sample	Original		Dominant[a]		Richness		Diversity	
	Reads	OTUs	Reads	OTUs	Chao1[b]	ACE[c]	Shannon	Simpson
N5	7974	394	7268	63	552	779	3.36	0.09
X7	6848	185	6494	32	424	615	2.21	0.29
X11	6889	424	6138	59	782	1249	3.05	0.16

[a] Dominant: OTUs defined by ≥10 reads in any sample are " dominant OTUs".
[b] Chao1: evaluation of species abundance.
[c] ACE: abundance-based coverage estimator.

The value of OTUs represented the species richness in a sample, and the Shannon index indicated the bacteria diversity in a sample that took into account the evenness of OTU distribution. As shown in Tab. 1, sample X11 had the highest bacteria richness, higher than in samples N5 and X7. However, sample N5 had the highest bacteria diversity, as demonstrated by a Shannon index of 3.36. Sample X11 had a relatively high Shannon index of 3.05. Sample X7 was low with both richness and Shannon index.

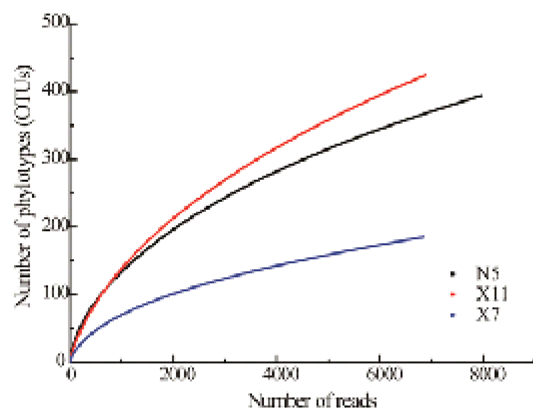

Fig. 1. Rarefaction analysis of the samples of N5, X7, and X11. Numbers of OTUs and reads were clustered at 97% sequence identity by default in QIIME, and rarefaction analysis was used the software of Analytic Rarefaction.

Differences in microbial communities in three production wells with different EOR processes

The microorganisms in produced waters collected from oil production wellheads are usually considered as those inhabiting the oil reservoirs. Microbial communities detected in three samples were compared between microorganisms in the three production wells with different EOR treatment processes.

Co-existing OTUs could provide substantial data to show the relationships of microbial communities in different environments. In this study, original OTUs and dominant OTUs for the microbial populations of N5, X7 and X11 were shown (Fig. 2). There were 50 co-existing OTUs detected in communities of the produced waters. 22 of 50 OTUs were significantly clustered into dominant OTUs. While, small percentages of

Fig. 2. Analysis of original OTUs and dominant OTUs for the samples. Venn diagram showed the unique and co-existing OTUs in the different libraries N5, X7, and X11

OTUs detected in the microbial community only in each produced waters were in the group of dominant OTUs (57 of 382 OTUs, 37 of 375 OTUs, and 13 of 143, respectively).

Based on the data of alignment, 23 different groups were identified within three samples.The microbial communities within the samples of N5, X7 and X11 showed similar 16S rRNA distributions at the phylum level with significant variations in numbers (Fig. 3A). The populations of Proteobacteria were predominant in samples N5, X7 and X11 and accounted for 98.54%, 75.18% and 81.35% of the number of obtained reads, respectively. This was followed by populations of Deferribacteres which represented 0%, 24.82% and 18.08% in the same samples, respectively. Roughly, most microbes in produced waters were mainly clustered into the phylum Proteobacteria. Similarly, some observations were reported that microbial populations inhabit oil reservoirs[3-7].

To further understand the differences in the important bacteria inhabiting the production wells, all dominant OTUs at the genus level were aligned and classified microbes into three groups only in sample N5, only in X7 and X11, and in all samples (Tab. 2). Those dominant groups only in sample N5 with the treatment of water-flooding

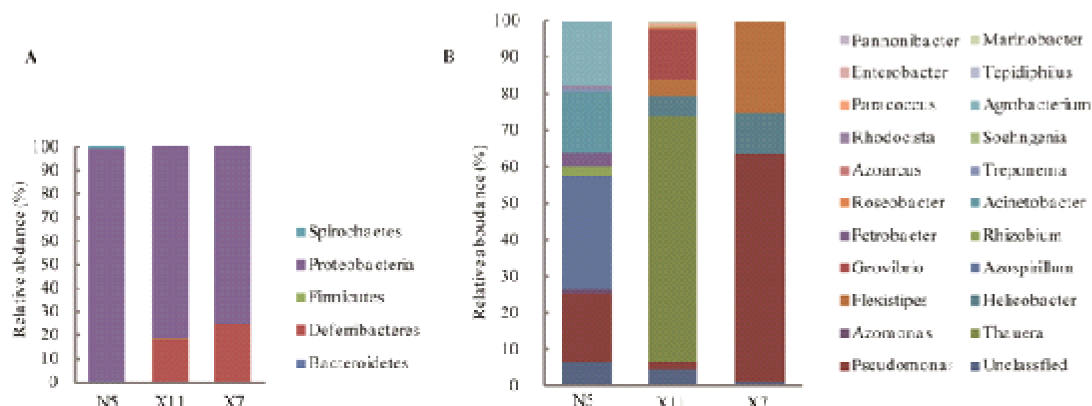

Fig. 3. Relative abundances of microbial community in clone libraries (N5, X7, and X11) at phylum (A) or genus level (B).

process were affiliated to *Azospirillum* (31.23%), *Agrobacterium* (17.38%), *Acinetobacter* (16.84%), *Petrobacter* (3.62%), which had abundance levels that were higher than 3.0%. However, these microbes were not detected in samples X7 and X11. The groups of *Azospirillum*, *Agrobacterium*, *Acinetobacter Petrobacter*, *Agrobacterium*, *Azomonas*, *Rhizobium*, *Rhodocista* and *Tepidiphilus* may be introduced by injecting water, because water-flooding has lasted for 38 years in the oil reservoir since 1974. The occurrence of new microbial communities in water-flooded oil reservoirs was ascribed to the injected water[2].In general, microbes detected in the water-flooded well would show some special characteristics in metabolism and had close similarity with those detected in the special environment of oil reservoirs due to the common characteristics of oil reservoirs having limited electron acceptors and donors and the large amount of hydrocarbons[2,17]. Consistently, the sequence affiliated with *Azospirillum* detected in soil polluted with crude oil could utilize aromatic hydrocarbons[18]. *Agrobacterium* was reported to removesulfur from sulfur-containing petroleum sample[19]. Members of the genus *Acinetobacter* also identified in monitored production wells could commonly produce bioemulsifier emulsan[20]. This genus of *Petrobacter* was isolated from higher temperature oil reservoirs[21, 22].

In comparison, the dominant reads detected only in X7 and X11 were aligned to *Thauera* (67.29% in X11, 0.05% in X7), *Helicobacter* (5.31% in X11, 11.01% in X7), *Flexistipes* (4.19% in X11, 24.82% in X7), and *Geovibrio* (13.90% in X11) (Tab. 2), which were not identified in sample N5. A possible containment for the introduction of *Thauera*, *Helicobacter*, *Flexistipes*, and *Geovibrio* to the production wells was mixed with the injected nutrients or polymers without sterilization. Most the detected microbes represented invariably close similarities with those detected in environments associated with oil. Denitrifying bacterium *Thauera* sp. was only detected in oil fields[23, 24], and be used alone or in concert with other microorganisms to improve oil recovery[25]. *Flexistipes* originated from a high temperature environment[26]. Members of *Geovibrio* were detected in the samples from oil reservoirs[7].

Dominant groups in all three production wells (N5, X7 and X11) belonged to *Pseudomonas*, *Enterobacter*, Unclassified bacterium. *Pseudomonas* were dominant in sample X7 with a percentage of 62.72%, while this was followed by those in the sample N5 (18.95%) and X11 (2.44%). Only a small percentage of reads (less than 0.5%) of this genus *Enterobacter* was also detected in three samples. Remarkably, members of the *Pseudomonas* and *Enterobacter* were frequently detected and isolated from environments associated with oil, indicated that the two genera possessed exceptional survival abilities in the environment of oil reservoirs after introduction into wellholes[27, 28].

Potential microorganisms and mechanisms of the field trial of oil recovery

Nutrient injection would result in an alteration of microbial communities in oil reservoirs

Table 2. Dominant microbes inhabiting only one produced water from water-fooding EOR process (N5), produced waters from both heterogeneous combination flooding (X11) and MEOR (X7) processes and from all the three production wells

Microorganisms detected								
In produced water from water-flooded well		In produced waters from well with heterogeneous combination flooding (X11) and well with MEOR (X7) process			In all samples			
Microbes	Abundance[a]	Microbes	Abundance[a]		Microbes	Abundance[a]		
	N5		X11	X7		N5	X11	X7
Azomonas	1.03	Thauera	67.29	0.05	Pseudomonas	18.95	2.44	62.72
Azospirillum	31.23	Helicobacter	5.31	11.01	Enterobacter	0.04	0.37	0.02
Rhizobium	2.7	Flexistipes	4.19	24.82	Unclassified	6.41	4.37	1.06
Petrobacter	3.62	Geovibrio	13.9	0				
Acinetobacter	16.84	Roseobacter	0.55	0				
Rhodocista	0.95	Azoarcus	0.16	0				
Agrobacterium	17.38	Paracoccus	0.37	0				
Tepidiphilus	0.19	Marinobacter	0.41	0.32				
		Pannonibacter	0.37	0				

Abundance showing the abundances calculated based on the numbers of reads.

(10,11). Microbial communities in the production well X7 after nutrient injection were compared with ones in water-flooded well N5.Firstly, the number of detected OTUs could provide a preliminary insight into the significant differences and relationships of microbial communities among the samples N5 and X7 (Fig. 2). 32 OTUs of sample X7 decreased significantly after the MEOR field trial, two times fewer than 63 ones of sample N5. Moreover, there were just 3 co-existing OTUs both in sample X7 and N5.

To gain in-depth insights into the microbes that were stimulated by the MEOR process, the microbial community in samples N5 and X7 was analyzed (Fig. 3B). Microorganisms with an obvious increase in the number might be potential for MEOR after nutrient injection. The number of reads for the OTUs affiliated closely with *Pseudomonas* increased sharply from 1364 (18.95%) to 4043 (62.72%) after nutrients injection(Tab. 2). This was followed by the reads of OTUs aligned to *Flexistipes* increased from 0 (0%) to 1612 (24.82%), and reads for OTUs clustered within *Helicobacter* increased from 0 (0%) to 715 (11.79%). It is well-known that strains of *Pseudomonas* could produce rhamnolipids that could improve mobility of crude oil[29], and was also applied to improve 20% of oil recovery in high-temperature oil reservoir[30]. Moreover, there were two groups of *Flexistipes* and *Helicobacter* that were too few to be detected in sample N5, were also activated. The groups of *Thauera*, *Helicobacter*, *Flexistipes*, and *Geovibrio*, which represented to be close associated with oilenvironment, may be introduced to the production well X7 with the injected nutrients without sterilization. Additionally, after nutrients were injected, the groups of *Azospirillum*, *Agrobacterium*, *Acinetobacter*, *Petrobacter*, *Rhizobium*, *Azomonas*, *Rhodocista*, *Treponema*, *Tepidiphilus*, and *Soehngenia*, detected only in the sample N5, were inhibited.The significant decrease of reads showed in the OTU aligned to *Azospirillum*, *Agrobacterium*, and *Acinetobacter* with a drop from 2245 (31.23%), 1263(17.38%), and1224(16.84%), to 0 (0%), and Unclassified bacterium with a decrease from 448(6.41%) to 69 (1.06%).

Without further isolation and metabolic analysis, microbial activities and products took place of the activated microbes during the process of MEOR, could be not determine specifically. Much less, microbial mechanisms for oil recovery act synergistically in the subsurface environments[2,31]. However, the analysis of microbial community could provide a clear picture for the importance of various microorganisms in the MEOR process which is of great importance in guiding further studies concerning the potential application of the microbes.

In conclusion,differences in microbes inhabiting three different types of samples from Ng3 zone of Shengli oilfield were ascribed to different enhanced oil recovery processes with or without the introduction of nutrients or polymerswithout sterilization. Based on the co-existing OTUs resident microbes, samples X7 and X11 had close relationships. Microorganisms detected in the present study showed close similarities with those detected in samples from oil reservoirs, which revealed a clear picture of microbes that were special to the microbial ecology of oil reservoirs.After the injection of nutrients, microorganisms could be easily activated, and some ones well suffered inhibition with a sharp decrease in amount, which provided a regular pattern in selectively stimulating potential microbes for MEOR *in situ*.

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REFERENCES

1. Magot, M.; Ollivier, B.; Patel, B. K., Microbiology of petroleum reservoirs. *Antonie. Van. Leeuwenhoek.,* 2000; **77** (2): 103-116.
2. Youssef, N.; Elshahed, M. S.; McInerney, M. J., Microbial processes in oil fields: culprits, problems, and opportunities. *Adv. Appl. Microbiol.*, 2009; **66**: 141-251.
3. Orphan, V. J.; Goffredi, A. K.; Delong, E. F.;

Boles, J. R., Geochemical influence on diversity and microbial processes in high temperature oil reservoirs. *Geomicrobiol.,* 2003; **20**: 295-311.

4. Pham, V. D.; Hnatow, L. L.; Zhang, S.; Fallon, R. D.; Jackson, S. C.; Tomb, J. F.; DeLong, E. F.; Keeler, S. J., Characterizing microbial diversity in production water from an Alaskan mesothermic petroleum reservoir with two independent molecular methods. *Environ. Microbiol*., 2009; **11**(1): 176-187.
5. Ren, H. Y.; Zhang, X. J.; Song, Z. Y.; Rupert, W.; Gao, G. J.; Guo, S. X.; Zhao, L. P., Comparison of microbial community compositions of injection and production well samples in a long-term water-flooded petroleum reservoir. *PLoSOne*., 2011; **6**(8):e23258.
6. Silva, T. R.; Verde, L. C. L.; Santos, E. V. N.; Oliveira, V. M., Diversity analyses of microbial communities in petroleum samples from Brazilian oil fields. International Biodeterioration & Biodegradation., 2012; 1-14.
7. Zhang, F.; She, Y. H; Chai, L. J.; Banat, I. M.; Zhang, X. T.; Shu, F. C.; Wang, Z. L.; Yu, L. J.; Hou, D. J., Microbial diversity in long-term water flooded oil reservoirs with different *in situ* temperatures in China. *Sci. Rep.,* 2013; **2**: 760.
8. Brown, L. R., Microbial enhanced oil recovery (MEOR). *Curr. Opin. Microbiol*., 2010; **13**(3): 316-320.
9. Marchant, R.; Banat, I. M., Microbial biosurfactants: challenges and opportunities for future exploitation. *Trends in Biotechnology.,* 2012; **30**: 558-565.
10. Zhang, F.; She, Y. H.; Ma, S. S.; Hu, J. M.; Banat, I. M.; Hou,D. J., Response of microbial community structure to microbial plugging in a mesothermic petroleum reservoir in China. Appl. *Microbiol. Biotechnol*., 2010; **88**(6): 1413-1422.
11. Zhang, F.; She, Y. H.; Li, H. M.; Zhang, X. T.; Shu, F. C.; Wang, Z. L.; Yu, L. J.; Hou, D. J., Impact of an indigenous microbial enhanced oil recovery field trial on microbial community structure in a high pour-point oil reservoir. *Appl. Microbiol. Biotechnol.,* 2011; **95**(3):811-821.
12. Kumaraswamy, R.; Ebert, S.; Gray, M. R.; Fedorak, P. M.; Foght, J. M., Molecular- and cultivation-based analyses of microbial communities in oil field water and in microcosms amended with nitrate to control H_2S production. *Appl. Microbiol. Biotechnol.*, 2011; **89**(6): 2027-2038.
13. Wang, J.; Ma, T.; Zhao, L.; Lv, J.; Li, G.; Zhang, H.; Zhao, B.; Liang, F.; Liu, R., Monitoring exogenous and indigenous bacteria by PCR-DGGE technology during the process of microbial enhanced oil recovery. *J. Ind. Microbiol. Biotechnol*., 2008; **35**(6): 619-628.
14. Lenchi, N.; Inceoglu, O.; Kebbouche-Gana, S.; Gana, M. L.; Lliros, M.; Servais, P.; Garcia-Armisen, T., Diversity of Microbial Communities in Production and Injection Waters of Algerian Oilfields Revealed by 16S rRNA Gene Amplicon 454 Pyrosequencing. PLoS One., 2013; **8**(6): e66588.
15. Zhang, F; She, Y. H.; Banat, M. I.; Chai, L. J.; Yi, S. J.; Yu, G. M.; Hou, D. J., Potential Microorganisms for Prevention of Paraffin Precipitation in a Hypersaline Oil Reservoir. *Energy Fuels.,* 2014; **28** (2): 1191-1197.
16. Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I.,, QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods.*, 2010; **7**: 335–336.
17. Kryachko, Y.; Dong, X.; Sensen, C. W.; Voordouw, G., Compositions of microbial communities associated with oil and water in a mesothermic oil field. *Antonie. Van. Leeuwenhoek.,* 2012; **101**(3): 493-506.
18. Anna, G.; Maria, K.; Andrzej, P., Bioremediation of crude oil derivatives in soils naturally and artificially polluted with the use ofmaize as the test plant. *Acta. Sci. Pol., Agricultura.,* 2010; **9**(3): 13-24.
19. Hou, Y. F.; Li, C. H.; Kong, Y.; Feng, L. J.,; Guo, N., Study on Growth Conditions and Degradation Ability of Biodesulfurizing Bacterium "*Agrobacterium tumefaciens*" UP-3. Engineering Our Future: Are We up to the Challenge? 2009; 27-30.
20. Chen, J.; Huang, P. T.; Zhang, K. Y.; Ding, F. R., Isolation of biosurfactant producers, optimization and properties of biosurfactant produced by *Acinetobacter* sp. from petroleum-contaminated soil. *J. Appl. Microbiol.,* 2012; **112**(4): 660-671.
21. Salinas, M. B.; Fardeau, M. L.; Cayol, J. L.; Casalot, L.; Patel, B. K.; Thomas, P.; Garcia, J. L.; Ollivier, B., *Petrobacter succinatimandens* gen. nov., sp. nov., a moderately thermophilic, nitrate-reducing bacterium isolated from an Australian oil well. *Int. J. Syst. Evol. Microbiol*., 2004; **54**(3): 645-649.
22. Yamane, K.; Maki, H.; Nakayama, T.; Nakajima, T.; Nomura, N.; Uchiyama, H.; Kitaoka, M., Diversity and similarity of microbial communities in petroleum crude oils produced in Asia. *Biosci. Biotechnol. Biochem.*, 2008; **72**(11): 2831-2839.
23. Hubert, C.; Voordouw, G., Oil field souring control by nitrate-reducing Sulfurospirillum spp.

that outcompete sulfate-reducing bacteria for organic electron donors. *Appl. Environ. Microbiol.,* 2007; **73** (8): 2644-2652.

24. Shartau, S. L.; Yurkiw, M.; Lin, S.; Grigoryan, A. A.; Lambo, A.; Park, H. S.; Lomans, B. P.; van der Biezen, E.; Jetten, M. S.; Voordouw, G., Ammonium concentrations in produced waters from a mesothermic oil field subjected to nitrate injection decrease through formation of denitrifying biomass and anammox activity. *Appl. Environ. Microbiol.,* 2010; **76**(15): 4977-4987.
25. Hendrickson, E. R.; Hendrickson, E. R.; Jackson, R. E.; Keeler, S. J.; Luckring, A. K.; Perry, M. P.; Wolstenholme, S., Identification, characterization, and application of *Thauera* sp. AL9:8 useful in microbially enhanced oil recovery. Patent: US7708065.,2010.
26. Lapidus, A.; Chertkov, O.; Nolan, M.; Lucas, S.; Hammon, N.; Deshpande, S.; Cheng, J. F.; Tapia, R.; Han, C.; Goodwin, L.; Pitluck, S.; Liolios, K.; Pagani, I.; Ivanova, N.; Huntemann, M.; Mavromatis, K.; Mikhailova, N.; Pati, A.; Chen, A.; Palaniappan, K.; Land, M.; Hauser, L.; Brambilla, E. M.; Rohde, M.; Abt, B.; Spring, S.; Goker, M.; Bristow, J.; Eisen, J. A.; Markowitz, V.; Hugenholtz, P.; Kyrpides, N. C.; Klenk, H. P.; Woyke, T., Genome sequence of the moderately thermophilic halophile *Flexistipes sinusarabici* strain (MAS10). *Stand. Genomic. Sci*., 2011; **5**(1): 86-96.
27. Das, K.; Mukherjee, A. K., Characterization of biochemical properties and biological activities of biosurfactants produced by *Pseudomonas aeruginosa* mucoid and non-mucoid strains isolated from hydrocarbon-contaminated soil samples. *Appl. Microbiol. Biotechnol*., 2005; **69**(2): 192-199.
28. Katsivela, E.; Moore, E. R.; Maroukli, D.; Strompl, C.; Pieper, D.; Kalogerakis, N., Bacterial community dynamics during in-situ bioremediation of petroleum waste sludge in landfarming sites. *Biodegradation*., 2005; **16**(2): 169-180.
29. Wang, Q.; Fang, X.; Bai, B.; Liang, X.; Shuler, P. J.; Goddard, W. A., Tang, Y., Engineering bacteria for production of rhamnolipid as an agent for enhanced oil recovery. *Biotechnol. Bioeng.,* 2007; **98**(4): 842-853.
30. Samir, M.; Abu El Ela, M.; Marsafy, S. E.; Tayeb, S. E.; Waly, A. A.; Sayyo, M. H., New Incubated *Pseudomonas aeruginosa* Bacteria for Increasing Oil Recovery under Reservoir Conditions. SPE 126104.
31. Maudgalya, S.; Knapp, R. M.; McInerney, M. J.; Nagle, D. P.; Folsmbee, M. J., Development of a Bio-Surfactant-Based Enhanced Oil Recovery Procedure. SPE 89473. 2004.