Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 13	No. 2	pp. 321-326	2014

Original Research Paper

Analysis of Microbial Community in Luliang Oil Field

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com
Received: 4-3-2014
Accepted: 6-5-2014

Key Words:
Luliang oil field
Microbial community
16S rDNA library,
Injection well
Oil well

ABSTRACT

This paper examines the total bacterial concentration and the functional bacteria concentration of the injection water from the injection well LU3064 and the produced fluid from the production well LU3036 using the plate count method and the most-probable-number method. We find that the bacterial community of the injection water is more complex and the bacteria concentration is higher than those of the produce fluid. Additionally, the surface tension of the produced fluid is lower than that of the injection water by 12%. We performed a diversity analysis to the injection water from LU3064 and the produced fluid from LU3036 by constructing the 16S rDNA clone library using molecular biology approaches. Through the comparison to GenBank, we found that the community in the injection water has a high diversity. The dominant bacteria belong to Alphaproteobacteria. The bacterial community of the produced fluid is relatively simpler, where *Pseudomonas* constitutes more than 80%, which are the dominant bacteria. There are also other functional bacteria such as *Bacillus* that exists in the produced fluid. We conclude that the oil recovery of Luliang oil field can be increased by microorganism flooding method.

INTRODUCTION

A certain time period after the water injection, microbial communities will be formed in the oil reservoir with relatively stable quantity and structure (Bao et al. 2004). The addition of nutrient activators and air can change the microbial community structure. The indigenous microorganism production technology is a comprehensive technique that increases the crude oil recovery rate by activating the microorganisms that produce metabolites which can increase the crude oil recovery using a selective control scheme (Brown Lewis 2010). Compared with exogenous microbial production technology, which injects the microorganisms to the stratum, indigenous microorganism production technology does not require large scale ground equipment on site. In addition, it is economic, environmental-friendly, and easy to implement. Therefore, it has become a new technology for oil recovery enhancement (Zheng et al. 2012).

The indigenous microorganisms in the reservoir adapt to special conditions such as high temperature and high pressure (Yamane et al. 2011). They also can slowly grow, reproduce, and metabolize under the special conditions. The key of the indigenous microorganism production technology is to activate the indigenous microorganisms in the stratum water that are useful to oil production by nutrient activators (Bao et al. 2011, Gao et al. 2011). Nutrient activators can be injected to the oil reservoir through water injection wells. It is possible to perform directional activation to microorganisms in the stratum water, which is beneficial to the enhancement of the recovery of crude oil. Understanding the bacterial communities in the injection water and the produced fluid helps the design of nutrient activators (Gao et al. 2013).

In this paper, the bacterial community of LU3064 injection water and LU 3036 produced fluid of Luliang oil field is examined using the 16SrDNA clone library (Zhang et al. 2010). We conclude that it is suitable to use microorganism flooding method to increase oil recovery in Luliang oil field.

MATERIALS AND METHODS

Main reagents and equipment: DNA polymerase, pEASY-T3 Cloning Kit, Trans1-T1 competent cells were all purchased from Beijing Transgen Biotech Co. Ltd.; ampicillin, IPTG and x-Gal were purchased from Biodee Company; primers were synthesized by Shanghai GeneCore Bio Technologies Co. Ltd.

Experimental water: Samples of injection water and production water used in the experiment were taken from the water-injection well LU3064 and oil well LU3036 of Luliang oilfield with a temperature of 37°C in Xinjiang,

China. The formation water of calcium chloride type was characterized by mineralization of 8471.2 mg/L.

Counting of bacteria, analysis of bacterial community and test of surface tension: The overall bacterial concentration in water sample LU3064 and LU3036 is examined by plate count method (LB culture medium: peptone 1%, yeast extract 0.5%, NaCl 1%, agar powder 1.5%, the pH value is adjusted to 7.5, autoclaving for 20 minutes). The concentrations of HOB, NRB, TGB, FMB and SRB of the samples were examined by the most-probable-number method (Wang et al. 2011). The surface tensions of the samples were measured by instrument FTA1000B.

DNA extraction: The water samples were filtered by gauze to exclude oil and sediment. They are centrifuged with 8000 rpm for 5 minutes; 800μL CTAB was added to the thallus and shaken up. The samples were heated in a 65°C water bath for 1 hr and then 800 μL chloroform/isoamyl alcohol was added to the samples and gently shaken. The samples were centrifuged for 10 minutes with 12000rpm under 20°C and supernatant was collected. The supernatant was well mixed with same volume of CI (chloroform : isoamyl alcohol = 24:1) and then centrifuged for 10 minutes with 12000 rpm, and the supernatant was collected. Same volume of precooled isopropanol was taken and supernatant was slowly added to it. The mixture stands overnight under -20°C. After that, it was centrifuged for 5 minutes with 10000rpm under 4°C and the supernatant is discarded. 1mL 70% ethanol was then added to the remainder and the mixture was centrifuged for 5 minutes with 10000 rpm under 4°C. The precipitate was washed twice and then washed using 1mL anhydrous ethanol once. The washed precipitate was air-dried for 30 minutes under 37°C and then dissolved in TE. DNA was examined by agarose gel electrophoresis.

DNA extraction: The amplification of 16S rDNA of bacteria in the water samples was done by consensus primers (8F,5'-AGA GTT TGA TCC TGG CTC AG-3';1541R,5'-AAG GAG GTG ATC CAG CC-3') (Gittel et al. 2009) and 1 U DNA polymerase, 0.5μL for each in a pair using the procedure described as follows: Predenaturation 5 minutes under 95°C; then denatured for 45s under 94°C, annealed for 45s and extended for 90s under 72°C, repeated for 30 times; then extended for 10 minutes under 72°C (Strapoc et al. 2008). The products of PCR reaction were examined by 1.2% agarose gel electrophoresis. The obtained 16S rDNA sections are connected with Peasy-T3 carrier and transformed to competent cells. Wait until clones are formed and randomly select 100 clones for verification using primer T7 (5'-TAA,TAC,GAC,TCA,CTA,TAG,GG-3') and SP6 (5'-ATT,TAG,GTG,ACA,CTA,TAG,AA-3') (Weert et al. 2011) to remove false positive clones. The clones are then sent to Beijing Aibo Yonghua Biotechnology Company for sequencing. The obtained 16S rDNA sequence was looked up from the GenBank.

The analysis of phlogeny: The positive plasmids were sequenced. Blast engine was adopted to figure out the bacteria which were as most similar with the sequences gained. A phylogenetic tree was constructed by software Bioedit and Mega4.0 using the neighbour-joining method (Yang et al. 2013).

RESULTS AND DISCUSSION

Counting of bacteria, analysis of bacterial community and test of surface tension: The total bacterial concentration and the concentrations of all kinds of functional bacteria in the injection water are larger than those in the produced fluid in Luliang oil field, as given in Table 1. Moreover, the bacterial community in the injection water is more complex than that in the produced fluid, which indicates that most of the bacteria in the injection water cannot adapt themselves to the stratum environment where the temperature is high but the nutrition level is low. The surface tension of the produced fluid is lower than that of the injection water by 12%, which is not a significant drop.

16S rDNA PCR: PCR products with size of about 1500bp, clear bands, and high specificity were obtained from the total DNA of the three samples through consensus primers 8F and 1541R amplification 16S rDNA, as shown in Fig. 1.

The construction and sequencing of 16S rDNA clone library: The clone library of the three samples was successfully obtained through the connect transformation of the amplification products. A hundred clones were randomly selected from a clone pool. The 16S rDNA sequence of successfully transformed clones is compared with the GenBank as shown in Tables 2 and 3. Clones with same sequence were defined as OTUs. The 92 clones in the injection water of LU3064 have 21 OTUs. The 89 clones in the produced fluid of LU3036 have 5 OTUs.

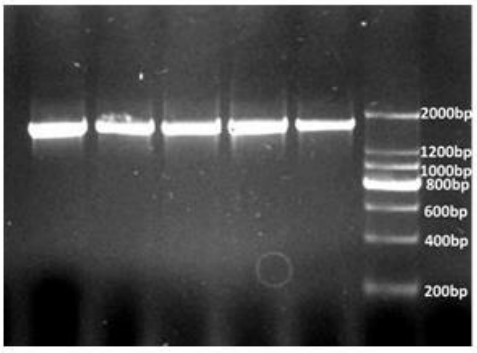

Fig. 1: The electrophoresis of 16S rDNA-PCR result.

Table 1: Numbers of microorganisms(sells/mL) and surface tension of samples.

Well number	organatrophs	HOB	SBR	NBR	FMB	TGB	Surface tension (dyn/cm)
LU3064	1.21×10^4	7.0×10^2	2.5×10^1	1.1×10^4	2.0×10^4	1.1×10^4	72.81
LU3036	6.5×10^2	0.6×10^1	0	2.5×10^2	2.5×10^1	1.4×10^4	62.13

Note: HOB = hydrocarbon-oxidizing bacteria; FMB = fermentative bacteria; NRB = nitrate-reducing bacteria; SRB = sulphate-reducing bacteria; TGB = Total Growth Bacteria

Table 2: Results of 16S rDNA gene clones isolated from LU3064 injected water.

No.	No. of clones involved	Length /bp	Register No.	Compared sequence		Homo-geneity
				Register No.	Known bacteria in the GenBank	
A1	2	1535	KF733610	NR_025327	*Planctomyces maris* strain 534-30	98%
A2	18	1477	KF733611	EU726271	*Stappia indica* strain B106 16S ribosomal RNA gene	99%
A3	2	1542	KF733604	NR_102783	*Bacillus subtilis* subsp. subtilis str. 168 strain 168	99%
A4	4	1455	KF733612	JX094172	*Brevundimonas basaltis* strain 02-9 16S ribosomal RNA gene	99%
A5	2	1478	KF733613	NR_044545	*Marispirillum indicum* strain B142	98%
A6	15	1459	KF733614	GU441680	*Rhodobacter* sp. Cr5-50 16S ribosomal RNA gene	97%
A7	12	1476	KF733615	NR_044216	*Rhizobium selenitireducens* strain B1	97%
A8	2	1479	KF059939	HQ652582	*Rhizobium* sp. p49(2011) 16S ribosomal RNA gene	99%
A9	3	1552	KF059948	NR_074945	*Desulfobacula toluolica* Tol2	97%
A10	1	1544	KF733605	KC485000	*Bacillus licheniformis* strain B3-15 16S ribosomal RNA gene	99%
A11	2	1517	KF733616	JN713308	Clostridiales bacterium canine oral taxon 141 clone PU008	97%
A12	8	1460	KF733617	AJ810841	Rhodobacteraceae bacterium 218 partial 16S rRNA gene	99%
A13	3	1475	KF733618	AM084032	*Sinorhizobium* sp. R-25067 16S rRNA gene, strain R-25067	96%
A14	2	1542	KF733606	HE774679	*Bacillus amyloliquefaciens* subsp. plantarum YAU B9601-Y2	99%
A15	2	1531	KF733620	HQ222278	*Acidovorax* sp. enrichment culture clone Van62	96%
A16	4	1459	KF059945	FN376425	*Roseovarius* sp. AMV6 partial 16S rRNA gene	98%
A17	3	1476	KF059946	NR_042277	*Phenylobacterium falsum* strain : AC-49	99%
A18	1	1475	KF059947	KC878323	*Labrenzia* sp. WP69 16S ribosomal RNA gene	99%
A19	2	1530	KF059938	NR_102806	*Thalassolituus oleivorans* MIL-1 strain MIL-1	99%
A20	1	1482	KF733619	FN397680	*Altererythrobacter* sp. AMV9 partial 16S rRNA gene	96%
A21	3	1496	KF059940	GQ902908	Acidobacteria bacterium enrichment culture clone Ac-F6	98%

Table 3 Results of 16S rDNA gene clones isolated from LU3036 produced water

No.	No. of clones involved	Length /bp	Register No.	Compared sequence		Homogeneity
				Register No.	Known bacteria in the GenBank	
B1	10	1477	KF733607	JQ396552	*Sphingomonas* sp. MN5-8 16S ribosomal RNA gene	98%
B2	68	1528	KF733608	JQ773431	*Pseudomonas aeruginosa* strain RI-1	99%
B3	6	1542	KF733604	NR_102783	*Bacillus subtilis* subsp. subtilis str. 168	99%
B4	1	1492	JX307094	AJ231186	*Planctomyces* sp. (strain 248) 16S rRNA gene	99%
B5	4	1529	KF733609	FJ859914	*Pseudomonas stutzeri* strain LC2-8	99%

Study on the diversity of microbial communities: The clone library results of the injection water LU3064 show that the clones in the injection water belong to 4 phyla and 8 classes. All OTU sequences with more than four clones belong to Alphaproteobacteria (73 clones in total). There are three OTUs belonging to Bacilli (5 in total). Besides, there are also Bateproteobacteria (2 in total), Deltaproteobacteria (3 in total), Gammaproteobacteria (2 in total), Clostridia (2 in total), Planctomycetacia (2 in total), and Acidobacteria (2 in total). The bacterial community of the produced fluid of LU3036 is simpler than that of the injection water. Only four bacterial genera were detected. They are *Pseudomonas* under

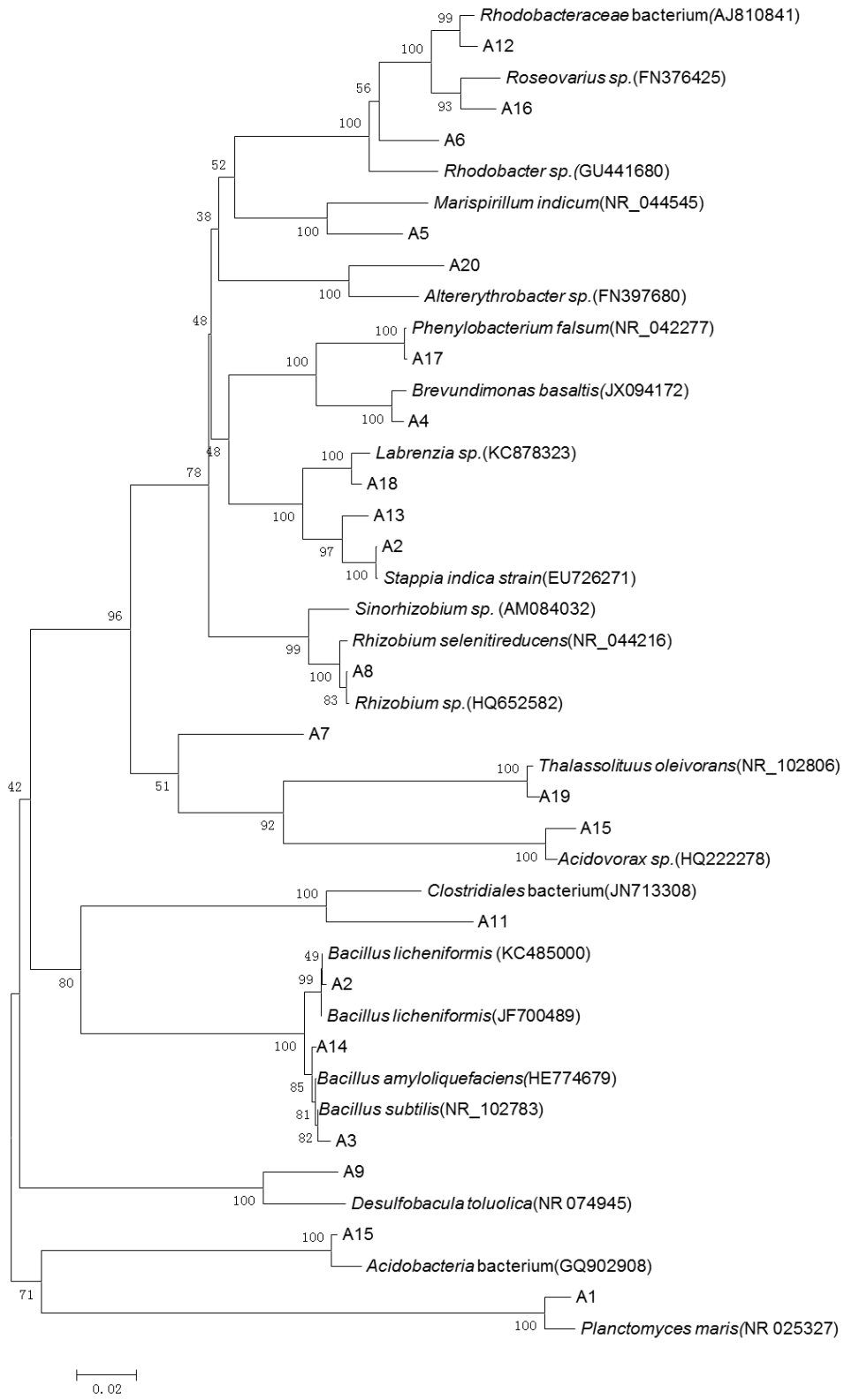

Fig. 2: The phylogenetic analysis of bacteria from LU3064 injected water.

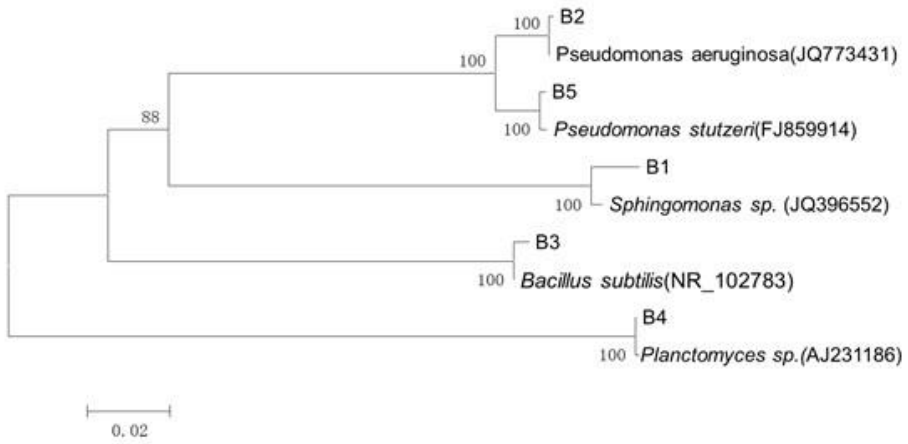

Fig. 3: The phylogenetic analysis of bacteria from LU3036 produced water.

Gammaproteobacterium (72 clones in total), *Sphingomonas* under Alphaproteobacterium (10 in total), *Bacillus* under Bacilli (5 in total), and *Planctomyces* under Planctomycetacia (2 in total) and both were detected in injection water as well.

The dominant bacteria in the water injection well, Alphaproteobacteria, do not have a high requirement on the water quality. They grow even when the nutrition level is low. A great amount of this kind of bacteria can be found in wastewater, but there are few reports on the oil production function of them. Most to the Betaproteobacteria are facultative heterotrophic bacteria that have both respiration and metabolism (Dou & Wang 2011). Bacilli and Gammaproteobacteria metabolize and degrade petroleum hydrocarbons and produce metabolites such as biological surfactants. They are oil functional bacteria that can be commonly seen in relevant reports. The *Pseudomonas* rhamnolipid produced by the metabolism belongs to Gammaproteobacteria. Clostridia ferment molasses in oil and produce carbon dioxide and organic acids, which support the enhancement of flooding. The analysis of the bacterial community of the injection water shows that although there is no report on the flooding enhancing ability of the dominant bacteria, the bacterial community of the injection water is rich and involves multiple oil functional bacteria. They can be activated by injecting nutrients and become dominant bacteria to play a role in the oil flooding and increase the crude oil recovery.

The *Pseudomonas*, *Bacillus* and *Sphingomonas* in the bacterial community of the produced fluid are commonly used oil functional bacteria (Xia et al. 2013). There is intensive research on the effect of *Pseudomonas* on the degradation of crude oil. They can be used to degrade long chain hydrocarbons, non-hydrocarbon, and aromatic hydrocarbons and produce Rhamnolipid biological surfactants (Xia et al. 2012, Wu et al. 2006). The *Pseudomonas* are widely applied to increasing the oil recovery and the remediation of oil pollution. The *Bacillus* are also widely-used hydrocarbon-degrading bacteria that produce surfactants (Simpson et al. 2011, Sung 2002). The metabolic components involve carbohydrates, lipids, and protein substances. It is reported that *Bacillus* are able to decrease the surface tension to 29.58mN·m^{-1}. In the microorganism flooding simulation experiments with high temperature and high salinity reservoirs, *Bacillus* increase the flooding by 5.7% compared to cases without *Bacillus* on average. *Sphingomonas* has an extremely wide range of metabolic capacity towards aromatic compounds. Additionally, some strains in the genus are able to synthesise valuable extracellular biopolymers.

In the produced fluid of Luliang oil field, it was detected that there were more than 80% *Pseudomonas* that are helpful to the increase of recovery, indicating that *Pseudomonas*, as commonly used oil functional bacteria in laboratories, are able to adapt themselves to the high pressure but low nutrition environment of the oil reservoir. It also confirms that *Pseudomonas* can grow and reproduce in the stratum with carbon as their only resource. Although the bacterial community of the produce fluid mainly consists of oil functional bacteria, the total number of bacteria is small. Therefore, the surface tension of the produced fluid is lower than that of the injection water, but not significantly. Overall, the oil recovery of Luliang oil field has the potential to be increased by microorganism flooding. The next step of the experimental task should focus on the design of activators that increase the concentration of *Pseudomonas* in the reservoir.

The analysis of phylogeny: MEGA4 program neighbour joining method was use to structure the phylogenetic tree.

The tree view program was used to output the phylogenetic tree, as shown in Figs. 2 and 3. By the phylogenetic tree, it could be found that the bacterial community of the injection water was more complex than produced water.

CONCLUSION

1. Compared with that of the injection water LU3064, the bacterial community of the produced fluid LU3036 had relatively simpler structure, and low total bacterial concentration. The surface tension of the latter was 12% lower than the former.
2. The bacterial community of the injection water was complex. The bacterial concentration is large. There existed multiple functional bacteria but none of them is the dominant bacteria. Eighty percent of the bacteria in the reservoir were *Pseudomonas*, which were the dominant bacteria. Besides, some commonly used oil functional bacteria such as *Bacillus* and *Sphingomonas* were also detected, but the bacterial concentration was low. As a result, the surface tension of the produced fluid was lower than that of the injection water, but the difference is little.
3. The examination of the bacterial communities of the injection water and the produced fluid of Luliang oil field showed that the bacterial community of the injection water is complex and involved more non-functional bacteria, though the natural selection of the conditions of the reservoir, only several functional bacteria that were helpful to the increase of oil recovery survived. This indicated that the oil recovery of Luliang oil field had the potential to be increased by the microorganism flooding technology.

ACKNOWLEDGEMENT

This project (2013AA064402) was supported by the National High Technology Research and Development Program 863-Microbial activation and simulation technology in the low-middle temperature reservoirs.

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