The Oil Recovery Mechanism of Activated Indigenous Microorganisms by Nutrient Agents

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ABSTRACT: In order to achieve a group of desired nutrient agent of indigenous microorganism of oil reservoir after polymer flooding in Daqing oilfield, biogas production experiments, physical simulation experiments and 16S rRNA gene library experiments were carried out. The first and second group of nutrient agents could make biogas pressure reach 0.85MPa and 1.15MPa in a high pressure vessel, respectively. The oil recovery efficiency of the first and second group of nutrient agents can be improved by 4.8% and 3.2% in physical simulation experiments, respectively. The major bacteria activated by the first activator system were Pseudomonas sp. 72%, Arcobacter sp. 18%, Thauera sp. 6%. The major bacteria activated by the second activator system were Thauera sp. 65%, Pseudomonas sp. 18%, Clostridium sp. 9%. Methanosaeta sp., Methanolinea sp., Methanobacterium sp. and Methanococcus sp. were the major archaea in oil reservoir after polymer flooding, which were not activated by the two activator systems.

After polymer flooding nearly 35% of the oil reserves has not been exploited, it is extremely difficult to further tap the potential remaining oil by some conventional techniques in oil reservoirs after polymer flooding[1,2]. Many foreign countries such as the United States, Russia and so on, have made lots of field tests of microbial enhanced oil recovery, and achieved some results[3,4]. China also carried out some research since the 1960's in some areas, have aslo achieved some results[5,6]. Microbial enhanced oil recovery technology as an important reserve technology, the experimental study has been carried out for many years in Daqing oilfield. But microbial enhanced oil recovery technology still stay in the field test stage. According to different microorganisms source, microbial enhanced oil recovery technology mainly includes the endogenous microbial oil recovery and the exogenous microbial oil recovery[7]. The endogenous microbial oil recovery refers to use the nutrients activator agents to activate microorganisms and improve productions of acid, biogas, biosurfactants, in order to improve oil recovery[8]. It has the advantages of good environmental adaptability, simple technical process, low cost and so on. The key of the technology is the screening tests of nutrients activator agents for specific reservoir environment. Biogas production performance tests, activated microbial numbers and types tests and physical simulation experiments were three important evaluation standards of the nutrient agents' activation effects[9].

Therefore, in order to test two groups of nutrient agents' activation effects for microorganisms of oil reservoirs after polymer flooding, biogas production experiments, physical simulation experiments and 16S rRNA gene library experiments were carried out. Biogas production experiments test biogas pressure in a high pressure vessel during the activation process. We constructed the 16S rRNA gene library to analyse the changes of bacteria and archaea before and after nutrient agents' activation. The oil recovery efficiency of nutrient agents system can be tested in physical simulation experiments. The study of biogas production performance and oil recovery mechanism of activated indigenous microorganisms by nutrient agents offer the reliable basis for the orientation of the endogenous microbial oil recovery.

MATERIALS AND METHODS

Materials

The experimental water was collected from the N2-D3-P40 well in the South Two Block of oil reservoirs after polymer flooding in Daqing oilfield, the experimental oil is dewatering crude oil of the N2-D3-P40 well in the South Two Block of oil reservoirs after polymer flooding in Daqing oilfield. Experimental equipments include stainless steel pressure vessel, MyCycler gradient PCR, incubator, physical simulation device, ultra low temperature freezer and so on.

Nutrient agents
The first group of nutrient agent: molasses 0.6%, $NaNO_3$ 0.1%, NH_4Cl 0.1%, yeast powder 0.02%, KH_2PO_4 0.02%, $MgSO_4$ 0.02%, K_2HPO_4 0.01%; the second group of nutrient agent: corn syrup powder 1.0%, $NaNO_3$ 0.2%, $(NH_4)_2HPO_4$ 0.15%, KCl 0.05%, $MgSO_4$ 0.02%。

Biogas production tests
Nutrient agents were dissolved in production water of the N2-D3-P40 well, put it into stainless steel pressure vessel, link pressure gauge to pressure vessel. The microorganisms of production water remained static culture at 45℃ about 25-30 days, recorded the biogas pressures during different periods.

Physical simulation experiments
The protocol of physical simulation experiments was described as[10]: (1) pumping vacuum; (2) saturating water; (3) saturating oil; (4) water flooding; (5) polymer flooding; (6) injecting nutrient agents; (7) subsequent water flooding; (8) the evaluation of the oil recovery efficiency.

Construction of 16S rRNA gene libraries
PCR amplification was performed with universal primers of 16S rRNA gene for bacteria and archaea[12,13]; the amplified 16S rDNA fragment was connected with pMD19-T vector (Promega); The ligated products were transformed into E.coli competent cells; Cells (50 μl), incubated for 45 min at 37 °C, were spread on LB plates containing ampicillin (100μg/ml), IPTG (50 mM), and X-Gal(80 μg/ml). About one hundred putative clones (white) from each plate were selected to sequence. The 16S rDNA sequences were analysed by GenBank database; the NJ method construction of phylogenetic trees used MEGA 4.1 software[14].

RESULTS AND ANALYSIS

The biogas production results of nutrient agents
Biogas production is one of some important mechanisms of the endogenous microbial oil recovery by injecting nutrient agents. The first and second group of nutrient agents could make the biogas pressure reached 0.85MPa and 1.15MPa in a high pressure vessel after 9 days, respectively (Figure 1). The biogas pressure's levels of the two systems were kept until 25 days. The results showed the two systems had good performance of the biogas production.
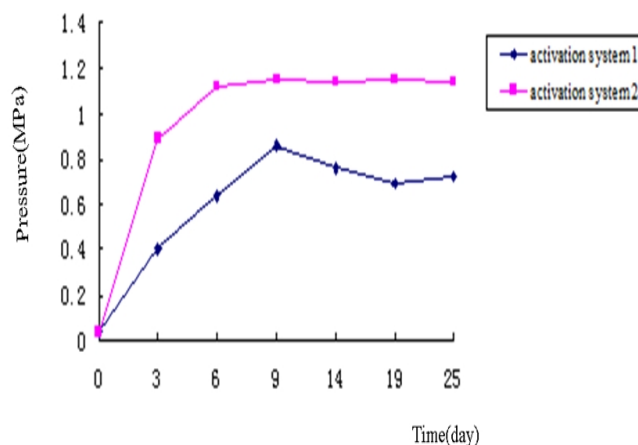
Figure 1. Pressure variation of high pressure vessels during the activation process of indigenous microorganisms

The effects of nutrient activators on microbial community structure

In order to analyse the activated microbial community by nutrient activators, 16S rDNA gene libraries of indigenous microorganisms were constructed before and after activator agents' activation. DNA sequences of the major dominant bacteria were compared with those in the NCBI database and related sequences are listed in Tables 1, 2 and 3.The major dominant bacteria are *Thauera* sp. (56%), *Pseudomonas* sp. (21%), uncultured *Arcobacter* sp. (8%)，*Acinetobacter* sp. (7%) and uncultured *Bacteroides* sp. (5%) before activation (Table 1). After the first activator agents' activation, the major dominant bacteria are *Pseudomonas* sp. (72%), uncultured *Arcobacter* sp. (18%) and *Thauera* sp. (6%) (Table 2). The major dominant bacteria are *Thauera* sp. (6%), *Pseudomonas* sp. (72%), uncultured *Arcobacter* sp. (18%) and after the second activator agents' activation (Table 3). Phylogenetic trees are shown in Figure 2, 3, 4.These results showed that the activated bacteria by the first group of nutrient agents are *Pseudomonas* sp. and *Arcobacter* sp., the activated bacteria by the second group of nutrient agents are *Thauera* sp., *Pseudomonas* sp. and uncultured *Clostridium* sp..

DNA sequences of the major dominant archaea were compared with those in the NCBI database and related sequences are listed in Tables 4, 5, 6. The major dominant bacteria are uncultured *Methanosaeta* sp. (54%), uncultured *Methanolinea* sp. (23%), *Methanobacterium* thermaggregans strain (9%) and *Methanococcus* sp. (7%) before activation (Table 4). After the two nutrient agents' activation, the major dominant archaea haven't been basically changed(Table 5, 6). The major dominant archaea are still uncultured *Methanosaeta* sp., uncultured *Methanolinea* sp., *Methanobacterium* thermaggregans strain and *Methanococcus* sp.. Phylogenetic trees are shown in Figure 5, 6.

The results of Physical simulation experiments

In order to assess the oil recovery effect of the activating endogenous microorganisms, physical simulation experiments were carried out. The results showed that physical simulation flooding could increase the oil recovery by 3.4% and 4.8% after the polymer flooding under the condition of 0.3 PV activator agents, respectively.
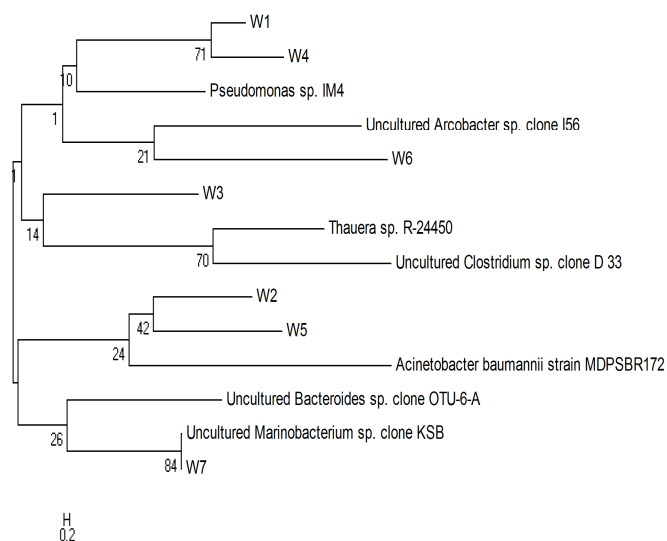
Figure 2. Phylogenetic trees of 16SrDNA gene sequences of bacteria before activation and their most similar sequences of GenBank
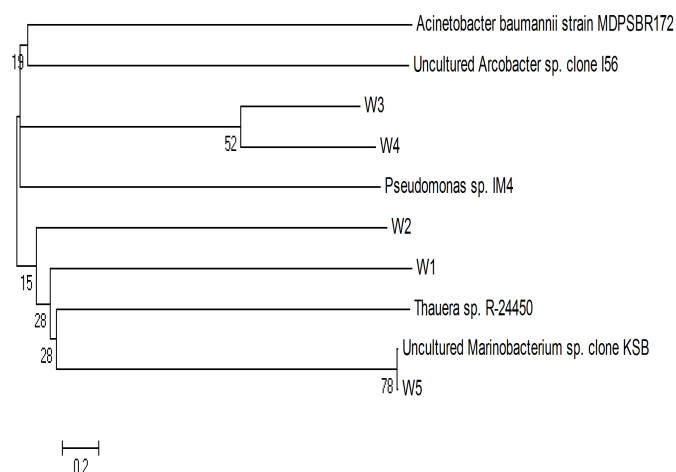

Figure 3. Phylogenetic trees of 16SrDNA gene sequences of bacteria after the first group of nutrient agents' activation and their most similar sequences of GenBank
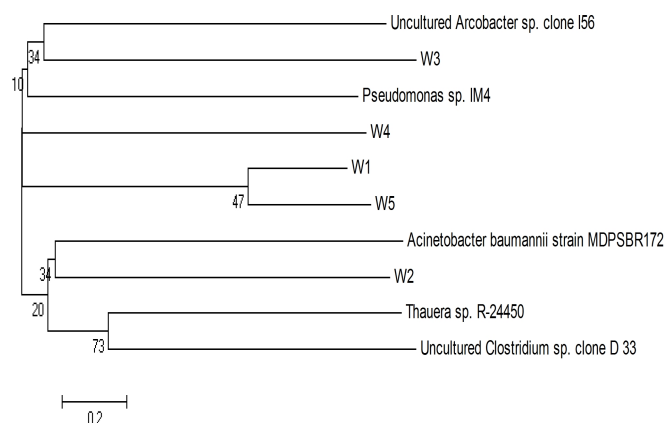

Fig.4 Phylogenetic trees of 16SrDNA gene sequences of bacteria the second group of nutrient agents' activation and their most similar sequences of GenBank
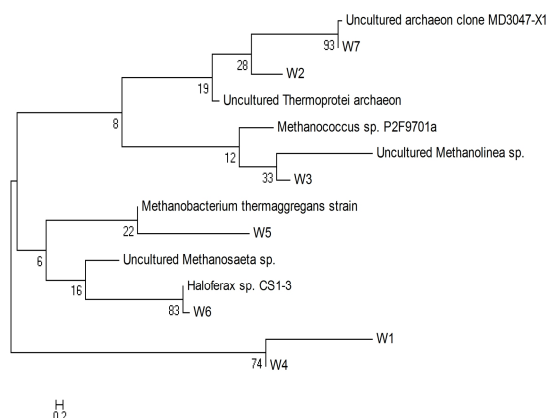
Fig.5 Phylogenetic trees of 16SrDNA gene sequences of archaea before activation and their most similar sequences of GenBank
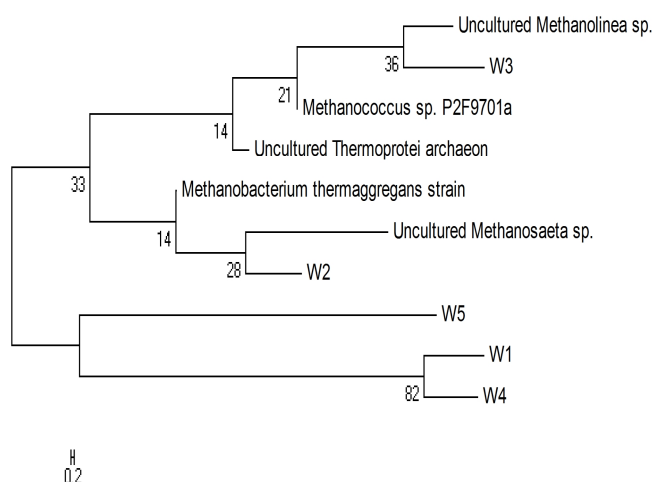

Fig.6 Phylogenetic trees of 16SrDNA gene sequences of archaea after the two nutrient agents' activation and their most similar sequences of GenBank

CONCLUSION

The second group of nutrient agents has better biogas production performance than the first group. Besides, the oil recovery efficiency of the first and the second group of nutrient agents can be improved by 4.8% and 3.2% in physical simulation experiments, respectively. The activated bacteria by the first group of nutrient agents are *Pseudomonas* sp. and *Arcobacter* sp.; the activated bacteria by the second group of nutrient agents are *Thauera* sp., *Pseudomonas* sp. and uncultured *Clostridium* sp.. After the two groups of nutrient agents' activation, the major dominant archaea haven't been basically changed. These results showed biogas production of activated bacteria including *Thauera* sp., *Pseudomonas* sp. and uncultured *Clostridium* sp. by the second group of nutrient agents was a key mechanism of indigenous microorganism flooding.

ACKNOWLEDGEMENTS

Table 1 Analysis of 16S rDNA gene library of bacteria before activation

Type	Clone number	GenBank Accession number	Phylogenetically closest related organism phylogenetically identified closest related organism	Similarity /%	Origina
W1	56	AM231040	*Thauera* sp. R-24450	99%	activated sludge
W2	21	FJ211165	*Pseudomonas* sp. IM4	99%	soil
W3	8	AY692044	Uncultured Arcobacter sp. clone I56	95%	reactor
W4	6	JF513192	*Acinetobacter* baumannii strain MDPSBR172c	96%	salt-affected soil
W5	5	JQ624314	Uncultured *Bacteroides* sp. clone OTU-6-ABB	97%	wastewater
W6	3	KM494506	Uncultured *Clostridium* sp. clone D_33	85%	sample
W7	1	KF206381	Uncultured *Marinobacterium* sp. clone KSB13	99%	hot springs

Table 2 Analysis of 16S rDNA gene library of bacteria after activation of the first group of nutrient agents

Type	Clone number	GenBank Accession number	Phylogenetically closest related organism phylogenetically identified closest related organism	Similarity /%	Origina
W1	72	FJ211165	*Pseudomonas* sp. IM4	99%	soil
W2	18	DQ234112	Uncultured *Arcobacter* sp. clone DS028	95%	mangrove
W3	6	AM231040	*Thauera* sp. R-24450	99%	activated sludge
W4	3	JF513192	*Acinetobacter* baumannii strain MDPSBR172c	96%	salt-affected soil
W5	1	KF206381	Uncultured *Marinobacterium* sp. clone KSB13	99%	hot springs

Table 3 Analysis of 16S rDNA gene library of bacteria after activation of the second group of nutrient agents

Type	Clone number	GenBank Accession number	Phylogenetically closest related organism phylogenetically identified closest related organism	Similarity /%	Origina
W1	65	AM231040	*Thauera* sp. R-24450	99%	activated sludge
W2	18	FJ211165	*Pseudomonas* sp. IM4	99%	soil
W3	13	KM494506	Uncultured *Clostridium* sp. clone D_33	85%	sample
W4	3	DQ234112	Uncultured Arcobacter sp. clone DS028	95%	mangrove
W5	1	JF513192	Acinetobacter baumannii strain MDPSBR172c	96%	salt-affected soil

Table 4 Analysis of 16S rDNA gene library of archaea before activation

Type	Clone number	GenBank Accession number	Phylogenetically closest related organism phylogenetically identified closest related organism	Similarity /%	Origina
W1	54	KJ877692	Uncultured Methanosaeta sp. Clone	98%	oilfield-produced water
W2	23	KF692508	Uncultured Methanolinea sp. clone	99%	oilfield-injected water
W3	9	NR_113572	Methanobacterium thermaggregans strain	99%	gas-associated formation water
W4	7	AF306670	Methanococcus sp. P2F9701a	94%	estuarine environment
W5	4	HM041917	Uncultured Thermoprotei archaeon clone	99%	petroleum reservoir
W6	2	GQ478061	Haloferax sp. CS1-3	99%	salt brine
W7	1	KJ131413	Duganella sp. ZLP-XI	86%	pteridophyte rhizosphere soil

Table 5 Analysis of 16S rDNA gene library of archaea after activation of the first group of nutrient agents

Type	Clone number	GenBank Accession number	Phylogenetically closest related organism phylogenetically identified closest related organism	Similarity /%	Origina
W1	62	KJ877692	Uncultured Methanosaeta sp. clone	98%	oilfield-produced water
W2	25	KF692508	Uncultured Methanolinea sp	99%	oilfield-injected water
W3	7	NR_113572	Methanobacterium thermaggregans strain DSM	99%	gas-associated formation water
W4	6	AF306670	Methanococcus sp. P2F9701a	94%	estuarine environment
W5	1	HM041917	Uncultured Thermoprotei archaeon clone	99%	petroleum reservoir

Table 6 Analysis of 16S rDNA gene library of archaea after activation of the second group of nutrient agents

Type	Clone number	GenBank Accession number	Phylogenetically closest related organism phylogenetically identified closest related organism	Similarity /%	Origina
W1	61	KJ877692	Uncultured Methanosaeta sp. clone A141-A1	98%	oilfield-produced water
W2	24	KF692508	Uncultured Methanolinea sp.	99%	oilfield-injected water
W3	8	NR_113572	Methanobacterium thermaggregans strain DSM	99%	gas-associated formation water
W4	6	AF306670	Methanococcus sp. P2F9701a	94%	estuarine environment
W5	1	HM041917	Uncultured Thermoprotei archaeon clone	99%	petroleum reservoir

Table 7 The results of indigenous microorganism in the physical simulation flooding experiment

Core Number	k_g md	S_{or} %	E_w %	0.5PV, E_p %	0.3 PV Activation System	E_{meor} %
38	1006	71.8	37.6	15.7	Injected the first activator agents' system, cultured for 37 days at 45°C, biogas production was observed.	3.4
47	1023	65.6	48.6	8.5	Injected the second activator agents' system, cultured for 37 days at 45°C, biogas production was observed.	4.8
69	1012	67.8	48.9	10.9	Injected oil-production water of the N2-D3-P40 well, cultured for 37 days biogas production was observed.	0.8

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