Growth and metabolism of an aerobic thermophilic *Geobacillus* strain under simulated composite extreme reservoir conditions

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**Abstract.** The growth and metabolism of an aerobic thermophilic *Geobacillus* strain DM-2 under simulated composite extreme conditions of the Ng3, Gudao Block, Shengli Oilfield (China) with sufficient oxygen and nutrition were investigated. The experimental device for simultaneously simulating the temperature, pressure, salinity, porosity, permeability, fluid characteristics and development status of the target reservoir was designed, where the air assisted flooding technology was used. Particularly, the oxygen demand was estimated according to biotic and abiotic oxygen consumption. Despite the relatively lower rates of microbial growth and metabolism, the strain was able to grow under these conditions with the cell density increased from 10^6 to 10^8 cells mL⁻¹, and CH₃COO⁻ produced at the concentration of about 400 mg L⁻¹. Aerobic microorganisms, such as *Geobacillus* sp., may be promising candidates for microbial enhanced oil recovery and oily sewage treatment technology.

1. Introduction

Microbial enhanced oil recovery (MEOR) has been considered as a promising tertiary recovery technology utilizing the microorganisms and/or their metabolic products for recovery of residual oil, with some problems surrounding its use needed to be resolved[1]. One of the problems, the metabolism and reproduction of the target microbes, still remains an issue rather than a highly desirable reality for the implementation of MEOR. As petroleum reservoirs are anaerobic environments, anaerobic hydrocarbon degradation is the prevailing mechanism for deep subsurface petroleum biodegradation[2]. Due to the relatively lower rates of growth and metabolism of anaerobic microorganism, most studies about MEOR have focused on the injection of facultative bacteria or the activation of stratal microflora[3,4]. Aerobic microorganisms involved in the MEOR have been reported[5-7] due to their distinct advantages of the quick reproduction and metabolism. Hydrocarbon-oxidizing bacteria are the important components of the aerobic–anaerobic microbial trophic chains resulted from petroleum transformation[5], depended on which the injection of water–air mixture or H₂O₂ aqueous solution and the air assisted flooding technology are able to regulate the geochemical activity of microorganisms to enhance oil recovery [3,5].
Although some researches have been conducted on the aerobic microorganisms that are resistant to high temperature of oil fields[6,8], they are mainly limited to the isolation, identification, taxonomy, growth and metabolism under few reservoir environmental factors such as temperature and salinities. However, reservoir is the composite extreme environment of high temperature, pressure, salinity, hydrocarbon toxicity and porous media in addition to poor nutrition and lack of oxygen. Thus, it is difficult to extrapolate the laboratory results into what is to be expected in the field. This is an important reason why aerobic MEOR has not gained credibility in the oil industry. The purpose of this study was to investigate the effects of simulated composite extreme conditions of reservoir on the growth and metabolism of a typical aerobic thermophilic Geobacillus sp. with sufficient oxygen and nutrition.

2. Experimental

2.1. Bacterium and medium

The bacterium designated as DM-2 was identified as a Geobacillus sp. with oil–waste bioremediation function[9]. The strain was preserved in the China General Microbiological Culture Collection Center with No.1565. The ion concentrations (mg L⁻¹) of produced water were Na⁺+K⁺ (2707), Ca²⁺ (80), Mg²⁺ (48), Cl⁻ (3836), HCO₃⁻ (1068), SO₄²⁻ (0) and CH₃COO⁻ (0). The water pH was 8.0. The culture medium with produced water contained (g L⁻¹) 30.0 sucrose, 2.0 NaNO₃, 1.35 KH₂PO₄, 3.65 Na₂HPO₄·12H₂O, 0.01 FeSO₄·7H₂O and 0.5 Yeast powder (pH 8.0).

2.2. Experimental device and method

The experimental device shown in Figure 1 was designed to simulate the temperature, pressure, salinity, porosity, permeability, fluid characteristics and development status of the target reservoir. The average measured porosity of the cores (length 16.00 cm, diameter 2.15 cm) was 38.0–38.6%, slightly higher than the reservoir average value of 33%, and the permeability 1800–2200 mD was in the reservoir range of 1500–2500 mD. The salinity, pH and other agents applied to the well were simulated by the produced water. The experiments were performed at 60 °C, in the reservoir range of 60–69 °C, and 8 MPa, close to the reservoir value of 10 MPa. The produced water and crude oil were taken as the simulation of formation fluid characteristics, and the development status was simulated approximately by the primary water flooding with produced water. The residual oil saturation of the saturated cores was 28.3–31.5%.

Figure 1. The schematic diagram of simulating composite reservoir conditions for bacterial growth study

To observe the bacterial cell growth, one core was opened every two days. Gas was gathered at the core outlet by water drainage method, and fluid samples at the outlet and inlet of core were collected by gas drive. The hemacytometer counting method was used for the bacteria counting. The pH of liquids was measured, and the CH₃COO⁻ content and the gas composition were monitored with HP6890 gas chromatography (Agilent Technologies, Santa Clara, USA). Three replicates were carried out, and the mean values were used as results.
2.3. Estimation of the injection–gas–liquid ratio

To the growth and metabolism of the aerobic strain DM-2, oxygen is one of the most decisive constraints, consumed by biotic and abiotic factors in the reservoirs. The abiotic oxygen consumption of the simulated experiments has been reported earlier[10]. The biotic consumption was estimated according to the literature[7]. Then the injection–gas–liquid ratio (IGLR, V/V) of the mixture facilitating bacterial growth and metabolism was estimated.

2.4. Pore volume of nutrient injection

To supply sufficient nutrition for the bacterial cell growth, the pore volume (PV) of injected nutrient at the balanced transport in the core was determined by injecting nutrient at the displacement rate of 0.5 mL·min⁻¹, and collecting samples at core outlet at regular intervals until values of concentration were unchanged. The concentration ratio of nutrient at core outlet and inlet (C_o/C_i) was calculated. Sucrose concentration was determined by the standard anthrone colorimetry method with CARY50 UV spectrophotometer (Varian, Palo Alto, USA), and the nitrate was analyzed with the AAIII nutrient auto-analyzer (Bran+Luebbe, Hamburg, Germany).

3. Results and discussion

3.1. Estimation of the injection–gas–liquid ratio

The bacterial biomass could be calculated according to the following molar relations[7]:

\[
\text{C: H: O = 1: 2: 1; C: N: P = 100: 10: 2}
\]

(1)

Combined with the assumption that 50% of the carbon is incorporated into bacterial biomass and 50% is respired to CO₂ during aerobic growth, these relations give rise to the following growth equation:

\[
\frac{25}{3} (\text{C}_{12}\text{H}_{22}\text{O}_{11}) + 50 \text{O}_2 + 5 \text{N} + \text{P} = \text{C}_{50}\text{H}_{100}\text{O}_{50}\text{N}_{5}\text{P} + 50 \text{CO}_2 + \frac{125}{3} (\text{H}_2\text{O})
\]

(2)

where sucrose is the carbon source for bacterial cell growth, and \(\text{C}_{50}\text{H}_{100}\text{O}_{50}\text{N}_{5}\text{P}\) is a typical example of biomass.

As shown in formula (2), the mass ratio of dried biomass and the consumed oxygen is about 1:1. A typical bacterium, expressed as \(\text{E. coli}\), has average cell length of about 2 μm and width of 0.5 μm, and the dry weight of a single \(\text{E. coli}\) cell is known about 2.8 \(\times 10^{-13}\) g. Because the cell shape of the DM-2 was 0.5–0.7 × 1.5–4.0 μm with little difference to the typical one, the dry weight of a single DM-2 cell and the corresponding oxygen consumption are assumed to be 2.8 \(\times 10^{-13}\) g. As the bacterial cell density in the culture medium was usually \(10^8–10^9\) cells mL⁻¹, a slightly higher value of \(10^{10}\) cells mL⁻¹ is supposed here, then the maximum biotic oxygen consumption per milliliter of bacterial liquid is 2.8 mg.

In the case of simulated experiments with no crude oil, the oxygen consumption per milliliter of sterile formation water is 3.98 \(\times 10^3\) mg[10]. Thus, the total oxygen consumption based on the quick propagation of the strain is about 2.8 mg. Then the corresponding oxygen volume at atmospheric pressure is 1.96 mL, and the air volume is 9.8 mL, which means the IGLR of 10:1.

As for the experiments with saturated cores, the residual oil saturation was about 30% after first water flooding. The oil–water ratio of 1:2 (V/V) in the simulation system is assumed, and the crude oil corresponding to 1 mL formation water is 0.5 mL, and 0.48 g based on the surface density of 0.954 g cm⁻³. Supposing 50% of oil phase contacted with water phase, the crude oil corresponding to 1 mL formation water is 0.24 g, corresponding to the oxygen consumption of 4.84 mg according to the literature earlier[10]. Thus, the total oxygen needed by 1 mL of formation water is about 7.64 mg, the plus of biotic bacterial factor of 2.8 mg and abiotic factors of 3.98 \(\times 10^3\) mg (formation water) and 4.84 mg (crude oil). The converted air volume at atmospheric pressure is 26.74 mL. That is, the IGLR of 30:1 is required to supply sufficient oxygen.
3.2. Pore volume determination of nutrient injection

The transport of sucrose and NO$_3^-$ reached the balance at 3 PV with C$_e$/C$_i$ of 0.93 and 1.0 in the oil–saturated core, and 4.5 PV with C$_e$/C$_i$ of 0.97 and 1.0 in the unsaturated core, respectively. Meanwhile, the faster balance achievement of nutrients in the oil–saturated core may be caused by the volume occupation of existed crude oil in the core. Since the concentrations of sucrose and NO$_3^-$ in the unsaturated core at 3 PV reached relatively high ratios of 0.83 and 0.87, respectively, capable of meeting the needs of cell growth, the displacement volume of 3 PV is chosen here.

3.3. Growth and metabolism experiments

As the porosity and permeability of cores were determined with the produced water instead of sterile saline, whether the bacteria grew in the cores was DM-2 was investigated firstly. Results of community analysis of the produced water showed no existence of aerobic bacteria such as saprophyte, hydrocarbon degrading bacteria and iron bacteria. As for the anaerobic bacteria, no nitrate reducing bacteria was found. The sulfate–reducing bacteria survived at 0.6 cells·mL$^{-1}$, and the methanogens existed at 50 cells·mL$^{-1}$. The N$_2$, O$_2$ and CO$_2$ in the gas from the cores S4 and S8 were detected with the volume fraction of 83.07%, 16.22%, 0.71% and 80.75%, 18.59%, 0.66%, respectively. No CO, H$_2$S and CH$_4$ were found in all experiments. So only the target bacteria were able to grow and propagate under aerobic conditions. Moreover, similar cell morphology was visualized from microscope to that in the shake flask experiments, which provides the further evidence.

Table 1 Growth and metabolism of the Geobacillus strain DM-2 in the oil–saturated and unsaturated cores under the simulated reservoir conditions

| Cores  | IGLR$^b$ (V/V) | Incubation time (d) | Core inlet | Core outlet |
|--------|----------------|---------------------|------------|------------|
|        |                |                     | Cell density (cells·mL$^{-1}$) | pH | CH$_3$COO$^-$ (mg·L$^{-1}$) | Cell density (cells·mL$^{-1}$) | pH | CH$_3$COO$^-$ (mg·L$^{-1}$) |
| S1     | 30:1           | 2                   | 5.0×10$^7$  | 7.23 | 55 | 3.5×10$^5$  | 7.95 | 28 |
| S2     | 30:1           | 4                   | 5.3×10$^8$  | 6.41 | 352 | 4.0×10$^7$  | 6.87 | 65 |
| S3     | 30:1           | 6                   | 2.7×10$^8$  | 5.93 | 435 | 3.8×10$^8$  | 5.58 | 390 |
| S4     | 30:1           | 8                   | 1.5×10$^8$  | 5.98 | 440 | 2.1×10$^8$  | 6.21 | 372 |
| S5     | 10:1           | 2                   | 6.0×10$^8$  | 6.93 | 79 | 2.0×10$^7$  | 8.05 | 30 |
| S6     | 10:1           | 4                   | 4.0×10$^8$  | 5.62 | 337 | 2.5×10$^8$  | 7.12 | 54 |
| S7     | 10:1           | 6                   | 2.5×10$^8$  | 6.12 | 420 | 5.0×10$^8$  | 6.43 | 213 |
| S8     | 10:1           | 8                   | 1.0×10$^8$  | 5.83 | 385 | 3.0×10$^8$  | 6.57 | 320 |

$^a$Oil–saturated cores (S1, S2, S3, S4), oil–unsaturated cores (S5, S6, S7, S8)

$^b$IGLR: the injection–gas–liquid ratio

The cell density of culture broth in the simulated experiments was (1–4) × 10$^6$ cells·mL$^{-1}$ (pH 8.0), and the values at core outlet were (2–4) × 10$^5$ cells·mL$^{-1}$ after the injection. As the strain could grow on hexadecane as the sole carbon source, and acetic acid was the only short–chain fatty acid among the metabolic products[9], the CH$_3$COO$^-$ was determined in the experiments with its initial value of 29 mg·L$^{-1}$ in the culture broth. The results revealed that the strain could grow and metabolize under simulated reservoir conditions. It began to grow after 2–day–old incubation at inlets and 4–day–old incubation at outlets (Table 1). The longer growth lag phase at outlets may be caused by the relatively low initial cell density of bacteria. Thus, sufficient inoculum density is essential for the functions of the strain performed in the oil recovery technique. Comparison with the shake flask experiments[11] shows longer growth lag phase (≥ 2 days) and lower rates of growth and metabolism, but similar growth trend, bacterial cell density first increased and then decreased with the increasing incubation time. It may be explained that the large production of acetic acid may perform the function of inhibiting or even killing the bacteria themselves. Furthermore, it was also the lower acid–producing rate and less bacterial biomass under simulated experiments that caused the longer stationary phase. Few differences between growth and metabolism of the strain in the oil–saturated and unsaturated cores revealed that the presence of residual oil had no influence on the bacterial cell growth (Table 1), in agreement with the finding of the strain with crude oil biodegradation potentials[9]. The bacterial
cell density, pH and CH₃COO⁻ content at the core inlet were quite different from those at outlet, indicating little impact of diffusion on the transports of microbes and their metabolites.

4. Conclusions

The Geobacillus strain DM-2 had a significant growth and effective metabolism under the simulated extreme reservoir conditions of Ng3, Gudao Block, Shengli Oilfield with sufficient oxygen and nutrient, despite the relatively lower rates of microbial growth and metabolite production. Aerobic microorganisms, such as Geobacillus sp., may be promising candidates for MEOR and oily sewage treatment technology. Therefore, further research in real reservoir conditions is necessary to fully elucidate the growth and metabolism of aerobic microorganism.

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