Microbial Treatment for Prevention and Removal of Paraffin Deposition on the Walls of Crude Pipelines

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Abstract Two bacterial strains, paraffin removal strain and biosurfactant-producing strain, named BHJ-1 and QFL-1, were isolated from oil production wells in Daqing oilfield of China. They were subsequently identified as *Bacillus cereus* QAU68 and *Bacillus subtilis* XCCX, respectively. As an indicator of the degradation paraffin, the inoculum concentration of BHJ-1 and QFL-1 were added in different proportions, the optimum proportion was 5:2. In this proportion the degradation rate of paraffin could reach 64 %, the prevention rate of paraffin could reach 55 %.

Keywords Paraffin · *Bacillus cereus* · *Bacillus subtilis* · Degradation

Microbial treatment method of paraffin deposition has been used as a sustainable replacement to conventional treatment methods (chemical, mechanical and thermal methods) [1, 2]. Industrial scale tests were accomplished in Jidong, Daqing, Liaohe and Zhongyuan oilfields in China [3], but there is no large-scale application by microbial treatment on the whole, the reasons for restricting the large-scale application are short of validity period and less efficiency. Adding special biosurfactant can lower the

interfacial tension as their primary activity [4, 5], increase the hydrocarbon concentration in the water [6, 7], therefore adding the special biosurfactant may be propitious for combination of crude oil and bacterial liquid, prolong the validity period, increase the efficiency of microbial treatment and paraffin deposition.

During this study two isolates, paraffin removal strain and biosurfactant-producing strain, named BHJ-1 and QFL-1, were isolated from oil production wells in Daqing Oilfield of China. BHJ-1 was isolated from enrichment salts medium (ESM) containing 0.5 % (w/v) of crude oil. The system was operated at the following parameters: temperature 37 °C, pH 7.2, incubation period 7 days. In order to isolate oil-utilizing microorganisms, 0.1 ml of culture supernatant was transferred every 24 h onto nutrient agar plates [8]. QFL-1 was isolated from ESM and it was cultivated at orbital shaking (180 rpm) at 37 °C for 72 h. The culture fluid was spread on the cetyl tri ammonium bromide-methylene blue agar medium and cultivated at 37 °C for 9 days. Then, the colonies of the halo were extracted, streaked on crude oil agar medium and cultivated at 37 °C for 9 days. After five times plate streak, the pure strains could be purified [9]. The isolates were stored in isosensitest broth at 4 °C and were plated in Isosensitest agar for further investigation [10]. The photo of scanning electron microscope is shown in Fig. 1. Phylogenetic tree was constructed and analysis was done by neighbour joining method through MEGA 5 software (Fig. 2). Partial sequencing with both primers revealed that the closest matches, determined by a BLAST search, corresponded to *Bacillus cereus* QAU68 (99 % similarity, BHJ-1) and *Bacillus subtilis* XCCX (99 % similarity, QFL-1), respectively [11, 12].

As an indicator of the degradation of paraffin, BHJ-1 and QFL-1 were added in different proportions. The

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Fig. 1 The photo of scanning electron microscope ($\times 5.0$ K). **a** BHJ-1, **b** QFL-1

Fig. 2 Phylogenetic tree based on the 16S rRNA gene sequencing, constructed by neighbour joining method. **a** The position of strain BHJ-1 and their nearest, **b** the position of strain QFL-1 and their nearest

Table 1 The degradation rate of paraffin after BHJ-1 treatment

Degradation time (d)	0	7	14	21	28
Quantity of wax before degradation (g)	3.0	3.0	3.0	3.0	3.0
Quantity of wax after degradation (g)	3.0	2.3	1.9	1.4	1.0
The degradation rate of paraffin (%)	0	23	37	53	67

Table 2 The degradation rate of paraffin after mixed bacteria treatment after 7 days

The proportion of BHJ-1 and QFL-1	1:1	2:1	3:1	3:2	4:1	4:3	5:1	5:2	5:3	5:4
The degradation rate of paraffin (%)	50	62	60	57	54	48	44	64	54	46

proportions between the inoculum concentration of BHJ-1 and the inoculum concentration of QFL-1 were selected as 1:1, 2:1, 3:1, 3:2, 4:1, 4:3, 5:1, 5:2, 5:3 and 5:4. The degradation rate of paraffin after BHJ-1 treatment is shown in Table 1, the degradation rate of paraffin after mixed bacteria group treatment is shown in Table 2. Table 1 shows that BHJ-1 had better removal paraffin effect, but slower removal paraffin time, the degradation rate of paraffin reached 67 % after 28 days. Table 2 shows the optimal proportion of BHJ-1 and QFL-1 was 5:2, the degradation

Table 3 The prevention rate of paraffin after microbial treatment

Bacterial strain	The weight difference of paraffin tube after 7 days (g)		The prevention rate of paraffin (%)
	Before microbial treatment	After microbial treatment	
BHJ-1	0.47	0.35	26
Mixed bacteria	0.47	0.21	55

rate of paraffin could reach 64 % after 7 days, compared to no adding QFL-1, the removal paraffin time was much faster.

By controlling the temperature difference in crude oil and paraffin tube, circulating pump was operated for 7 days, which made paraffin deposit on the paraffin tube. The prevention rates of paraffin were measured before and after microbial treatment (Table 3). Table 3 shows that the prevention rate of paraffin could reach to 55 % after mixed bacteria treatment, higher than that after BHJ-1 treatment.

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