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## Selection of a heat-resistant oil recovery bacteria

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## Selection of a heat-resistant oil recovery bacteria

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**Abstract.** The core factor of Microbial Enhanced Oil Recovery (MEOR) is using the way of microbe growth and metabolic activity in the reservoir as well as microbial fermentation metabolic product to enhance crude Oil Recovery. The main work accomplished in this paper are as follows: according to the characteristics of geological environment and mineral composition of Changqing oilfield, separate and enrich strains which can tolerate 45 °C environment temperature and have the ability of normal growth and metabolism

### 1. Introduction

Microbial Enhanced Oil Recovery (MEOR) is a special method connect specific strains of bacteria and nutrients or metabolites with Oil Recovery<sup>[1]</sup>. This technology is a comprehensive way that can improve the recovery rate rapidly beside the traditional methods such as physical flooding and chemical flooding, and is an innovative application of new biological technology in the field of energy exploration and development<sup>[2]</sup>. It has the advantages of low construction cost, resource saving, high yield, quick effect, wide application range and environmental friendliness.

Countries represented by the United States and Russia in the field of MEOR research and application in oil field. In the early 20th century Beckman proposes the idea that the use of microorganisms for oil extraction<sup>[3]</sup>, in 1963, the Soviet scientists Kuznetsov proposed reservoir of endogenous microorganisms in oil reservoir can be crude anaerobic decomposition gases such as CO<sub>2</sub>, CH<sub>4</sub> and H<sub>2</sub> theory, the theory for the development of reservoir provides theory basis for endogenous microbial oil recovery technology<sup>[4]</sup>. In the 1990s, Russia conducted large-scale field tests in the Siberian plain and other oil fields<sup>[5]</sup>, while other countries successively carried out related research on microbial oil production<sup>[6]</sup>. Compared with other western countries, China was late to study on this region but developing rapidly<sup>[7]</sup>. Due to the rapid development of science and technology, microbial oil recovery technology is also obtained fast development, and has obtained the very good application effect. Changqing oilfield which located in Ordos basin, and the reservoir classification is belong to high-temperature reservoir which temperature range from 45 to 90 °C. In this paper, based on the characteristics of geological environment and mineral composition of Changqing oilfield, refer to other past cultivation from high temperature environment in reservoir heat resistant microorganisms used method. Field mine water and sludge which from the Changqing oilfield separate and screen out the strain which can tolerate the high temperature environment, Design a method to separate the heat resistant microorganisms, enrichment and domestication, screening and separation method of



separation of microbial strains which can produce thermal environment, and for subsequent heat anaerobic strains of microorganism resources research experience.

## **2. experimental materials and methods**

### *2.1. experimental materials*

*2.1.1. medium.* Enrichment medium of medium of high temperature resistant bacteria; Isolation and preservation medium of high temperature resistant bacteria; Emulsifying crude oil medium.

*2.1.2. samples.* 5 ore water samples (willow 18, Y 3, Y 5, L5, L6) and one mud (020).

*2.1.3. instruments and equipment.* Super clean table sw-cj-1f type: antai, suzhou; Constant temperature incubator lrh-250f type: Shanghai baidian; PH ub-7: DENVER INSTRUMENT; Constant temperature shaking machine: zhyi-2102c Shanghai zhicheng

### *2.2. experimental method*

*2.2.1. domestication and cultivation of medium-high temperature resistant strains.* Save the ore water sample and mud in the constant temperature shaking machine (45°C), And then transfer to the enrichment medium at a percentage of 1% capacity, which use crude oil as the only carbon source. Keep shaking in the constant temperature shaking machine under the condition of 45°C for 6 days.

*2.2.2. Isolation of target bacteria.* Set 5 gradients, the culture solution was uniformly coated on the separation medium by tablet gradient dilution method, Put the culture medium in 45 °C constant temperature incubator. Observed the colony morphology after 24 hours. List the edges, colors, shapes and moisture of the bacteria colony. Select single and suitable bacterial colonies and transfer to broth slant medium. After culture the bacteria 24 hours under the same condition. Number them and store in 4 degree refrigerator.

*2.2.3. screening of bacteria resistant to high temperature.* In order to select the bacteria with excellent performance in one step forward, we need to verify their two indicators. The first is the ability to produce surfactant, and the second is the ability to emulsify crude oil. For this experiment, the specific operation is to observe the size of oil drainage circle (we use a scale to measure the diameter of the oil drainage circle) and the effect of bacteria on crude oil emulsification experiment, the crude oil medium is called GJ medium, which can be judged the effect of bacteria on crude oil by the change of color.

## **3. Result and discussion**

### *3.1. Morphological identification of bacteria colony*

After shaking culturing under the condition of 45°C of the sample (L5, L6, Widow 18, Y3, Y5) and the sample of the mud. Distract the culturing liquid in the medium by the method of gradient dilution. And screen the bacteria group after 24 hours' culturing. And pick the proper bacteria. And name them, which is regard as a initially separated bacteria. The morphological observation of the isolated bacteria on the medium shown in Table 1.

**Table 1.** Result of morphology of 45 °C resistant bacteria

sample	edge	colour	shape	surface
020	Small wave	Ivory white	hump	dry
widow18	zigzag	Ivory white	hump	dry
Y3	Small wave	Ivory white	hump	dry
Y5	Small wave	Ivory white	hump	dry
L5	No rules	Ivory white	hump	moist
L6	Large wave	white	flat	dry

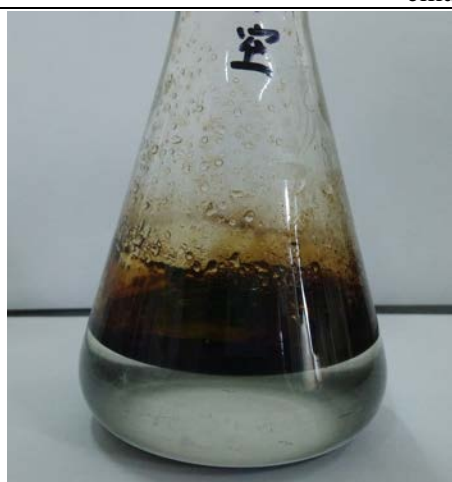
### 3.2. Preliminary screening results of high temperature resistant bacteria

#### 3.2.1. Crude oil emulsification results

The strain was added to the emulsified medium and incubated in constant temperature shaking flask (in the shaking machine) at 45 °C for 6 days. The sample was taken out for observation and compared with the blank control. The recorded results were as shown in Table 2 and figure 1-4:

**Table 2.** Effect of emulsification of crude oil

sample	The color of oil	Final emulsified state of crude oil
control	black	No change
020	puce	There is emulsification, liquid phase turbidity is good
widow18	black	No obvious emulsifying effect
Y3	black	The oil is dispersed into black granules, but there are still layers of oil and water.
Y5	black	No obvious emulsifying effect
L5	brown	Liquid phase becomes brown color turbid liquid, oil phase is dispersed into small droplets, emulsification is obvious.
L6	brown	Liquid phase changed into brown color turbid liquid, emulsification is obvious

**Figure 1.** blank control**Figure 2.** 020 oil emulsification effect

**Figure 3.** L5 oil emulsification effect**Figure 4.** L6 oil emulsification effect

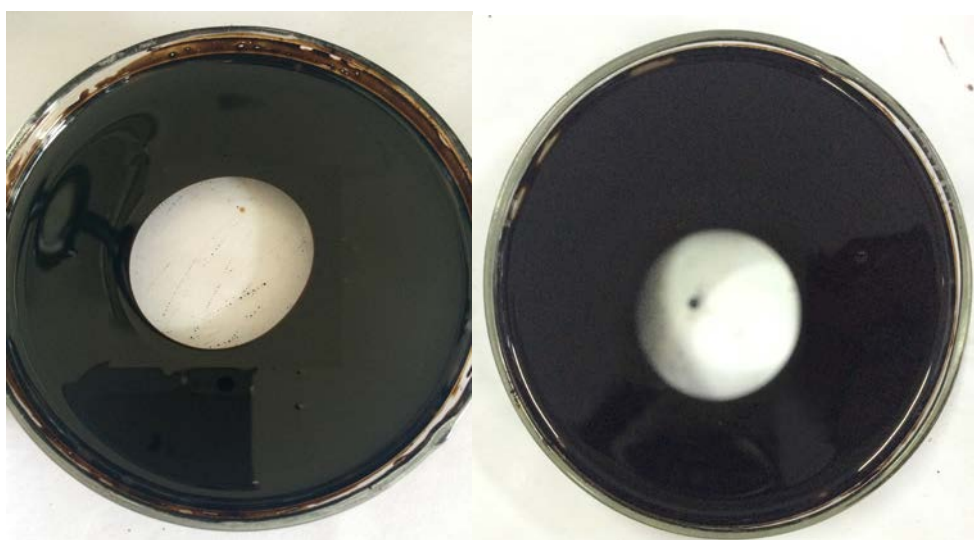
Compared with the blank control, The three strains with the best emulsifying effect are L6、 L5 and 020. The three strains can not only grow normally at 45 °C, but also have the ability to emulsify crude oil.

### 3.2.2. Screening results of Surfactant producing strains

The fermentation broth of each strain was measured the diameter of the oil drainage circle after 48 hours of shaking in high temperature. The measurement of the oil drainage circle is shown in Table 3 and figure 5.

**Table 3.** measurements of oil drainage circle

sample	Diameter (cm)		sample	Diameter(cm)
020	3.5	4	L5	0.3
Widow18			L6	0
Y3	0		Y5	0

**Figure 5.** Results of oil drainage circle measurements (from left to right are Widow18 and 020)

## 4. Conclusion

The three strains L6, L5 and 020 had excellent results in emulsifying crude oil experiment, while the fermentation broth of Widow18 and 020 had large oil drainage circle. Comprehensive analysis shows

that strain 020 performs well in oil drainage circle test and emulsified crude oil experiment. In the end, the target bacteria selected by this experiment were 020. The next work is to study and explore the culture conditions and growth characteristics of the strain, as well as to carry out the relevant 16SrDNA sequencing work.

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