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The effects of environmental conditions on the growth of petroleum microbes by microcalorimetry

Li Yu[a], Xingen Hu[a], Ruisen Lin[a,*], Honglin Zhang[b], Zhaodong Nan[b], Fenghua Li[b]

[a]*Department of Chemistry, Zhejiang University, Hangzhou, Zhejiang 310027, PR China*
[b]*Department of Chemistry, Qufu Normal University, Qufu, Shandong 273165, PR China*

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Abstract

The growth and metabolism ability of two strains of petroleum microbe isolated from oil reservoirs was studied by calorimetry. Thermal power–time curves under various environmental conditions (including temperature, acidity, salinity and carbon source) were determined. Typical microbial growth thermal power–time curves were obtained. By fitting the curves mathematically with the 'three-point method' under an inhibitory condition, the growth rate constants and the optimum growth conditions (including temperature, acidity and salinity) of the microbes studied were obtained. Irregular thermal power–time curves were obtained when the petroleum microbe E grew in the media containing different carbon sources. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Petroleum is a non-living resource, and its recovery is a global problem. Microbial enhanced oil recovery (MEOR) has many merits such as simple technology, convenient operation and low cost [1–3], and has attracted increasing attention. MEOR increases oil production by use of microbial activities and metabolic products [4]. Obtaining a microbial product involves finding and/or genetic construction of a producing strain [5], establishing optimal conditions for fermentation, purifying the product, and delivering it to the oil well for injection. Many of these steps could be avoided if the culture could be grown in the reservoir and the product formed in situ. Since few of the environmental parameters of reservoirs can be manipulated, it is necessary to find microbes that can grow and produce the desired metabolites under reservoir conditions of temperature, acidity, salinity and carbon source [6]. These conditions also place limitations on the reservoirs where microbes can be used for in situ treatment. Thus, it is necessary to study the effects of these conditions on the growth of petroleum microbes.

Microcalorimetry is an important tool for measuring metabolic activities of cells and biological tissues [7]. Heat production in microorganisms is due to biochemical reactions, so the growth of microorganisms can be studied with a continuous recording microcalorimeter in the same way as thermal phenomena due to chemical reactions [8]. The time depen-

* Corresponding author. Fax: +86-571-7951846.

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dence of the observed thermal effect (the thermal power–time curves) reflects changes in growth [7,9–18].

In this paper, the effects of temperature, acidity, salinity and long-chain alkanes on the growth and metabolic activities of two strains of petroleum microbe were investigated. Thermal power–time curves under various environmental conditions were determined by microcalorimetry. By fitting them with the 'three-point method' microbial growth model under an inhibitory condition, the optimum growth conditions were obtained. In addition, the thermal power–time curves of petroleum microbe E in the media containing different long-chain alkanes were determined. The irregularity of the thermal power–time curves obtained was preliminarily probed.

2. Experimental

2.1. Material

2.1.1. Strains and conditions of maintenance

The two strains of petroleum microbe studied, labeled E and F, were isolated from Chinese Shengli oil reservoirs and provided gratuitously by Life Scientific Institute of Shandong University (China). They were maintained as spore suspensions in the glucose medium A (see further) at 4°C in a biochemical culture cabinet. Exponentially growing cells were used as inocula for the experiments. Preparation of the inocula was made in an inoculation cabinet.

2.1.2. Media

The glucose and alkane media were prepared volumetrically as follows:

Medium A contained per 100 ml, NaCl (0.5 g), $(NH_4)_2SO_4$ (0.1 g), $MgSO_4{\cdot}7H_2O$ (0.025 g), $NaNO_3$ (0.2 g), $K_2HPO_4{\cdot}3H_2O$ (1 g), KH_2PO_4 (5 g), yeast extract (0.1 g), glucose (2.0 g); its pH was 7.00.

Medium B contained per 100 ml, 50 ml 0.1 M KH_2PO_4, different volumes of 0.1 M NaOH, and other ingredients the same as medium A; its pH ranges from 5.29 to 7.33.

Medium C contained per 100 ml, different amounts of NaCl (in this paper, '*w*' represents the concentration of NaCl), and the other ingredients the same as medium A; its pH was 7.00.

Medium D contained per 100 ml, the same inorganic ingredients as medium A, 0.4 ml Tween80 solution (2%,v/v) and different long chain alkanes: *n*-dodecane (2%, v/v), *n*-tetrasdecane (2%, v/v), *n*-hexadecane (2%, v/v) and *n*-octane (2%, v/v); its pH was 7.00. Tween80 is a surfactant that does not stop microbial growth. It incorporates the alkane into aqueous media as a clear, homogeneous phase.

All the media were sterilized at 120°C for 30 min. The same batch of medium and propagation microbe were used in parallel experiments.

2.2. Instruments

The calorimeter was a model 2277 Thermal Activity Monitor (Thermometric, AB, Sweden) operated in the stop-flow mode.

A glass electrode and pH-meter were used (mode HM-20s, TOA Electronics Ltd., Japan) to measure pH.

2.3. Methods

The complete cleaning and sterilization procedure for the flow tubing was as follows:

1. Sterilized distilled water was pumped through the system for 30 min at a flow rate of 30 ml/h.
2. HCl of 0.1 mol/l was pumped through the system for 30 min at a flow rate of 30 ml/h.
3. NaOH of 0.1 mol/l was pumped through the system for 30 min at a flow rate of 30 ml/h.
4. Alcohol solution (75%, v/v) was pumped through the system for 30 min at a flow rate of 30 ml/h.

Once the system had been cleaned and sterilized, sterilized distilled water was again pumped through the system at a flow rate of 30 ml/h for 30 min and the baseline determined. After a stable baseline was obtained, the microbial sample containing 5×10^5 cells/ml was pumped into the flow tubing at a flow rate of 30 ml/h. When the flow vessel was full, the pump was stopped, and the monitor began to record the thermal power curve of microbial growth. The microbial growth was considered to be complete when the recording pen returned to the baseline and became stabilized. Conversely, the irregular thermal power–time curves of microbe E in the medium D were followed continuously for at least 100 h.

3. Results and discussion

3.1. Growth in glucose medium

The thermal power–time curves of two strains of petroleum microbe growing in glucose medium (A, B and C) have been obtained. Here take microbe E as an example. Its thermal power–time curves under various environmental conditions are shown in Fig. 1. The experimental results demonstrate that the thermal power–time curves are highly reproducible under the same cultivation conditions.

For non-inhibitory conditions, the model of microbial growth follows an exponential law [19]

$$\frac{dN}{dt} = kN \tag{1}$$

For inhibitory conditions, the model of microbial growth follows the logistic equation [20]. In the growth phase, microbial number and time are related according to

$$\frac{dN}{dt} = kN - \beta N^2 \tag{2}$$

where N is the number of microbes at time t, k is the growth rate constant and β is the deceleration rate constant. If the thermal power–time curve is determined under isothermal and isometric conditions, where the supply of nutrients and dissolved oxygen are limited and the feedback inhibitory effect of the product also exists, the growth process can be fitted by the logistic equation. By integrating and arranging

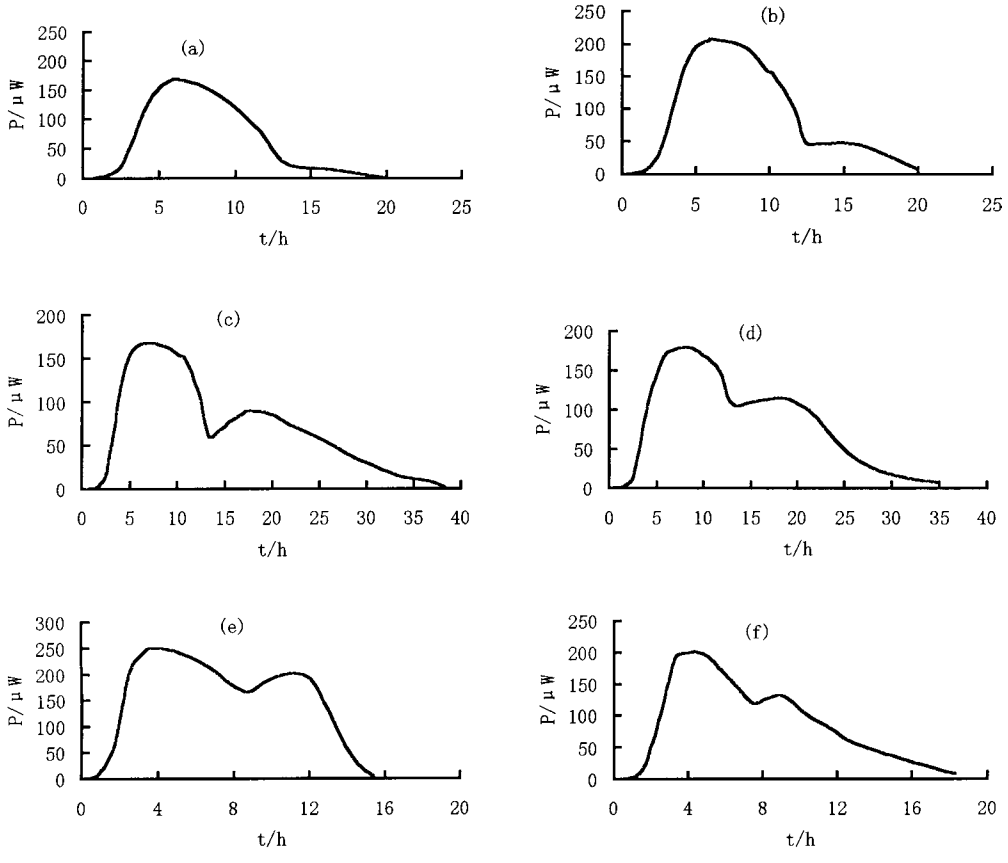

Fig. 1. Power–time curves of petroleum microbe E under various environmental conditions in the: (a) medium A (pH=7.00, w=2%, T=323.15 K); (b) medium A (pH=7.00, w=2%, T=318.15 K); (c) medium B (pH=5.63, w=2%, T=323.15 K); (d) medium B (pH=6.06, w=2%, T=323.15 K); (e) medium C (pH=7.00, w=2%, T=323.15 K); (f) medium C (pH=7.00, w=4%, T=323.15 K).

Table 1
P_t^*, P_t^{**} (in μW) and t at 318.15 and 323.15 K for microbe E[a]

318.15 K			323.15 K		
t (min)	P_t^*	P_t^{**}	t (min)	P_t^*	P_t^{**}
50	1.5	1.47	50	1.5	1.65
100	6.0	5.96	100	7.8	7.45
150	21.6	22.43	150	30.9	30.5
200	69.9	66.21	200	92.1	92.08
250	123.0	124.31	250	162.0	163.43
300	154.5	157.37	300	195.6	196.32
350	168.6	168.07	350	205.5	205.25

[a] P_t^* is experimental data; P_t^{**} is calculated data from Eqs. (7) and (8).

Eq. (2), we obtain

$$\ln \frac{V-N}{N} = a - kt \tag{3}$$

where a is a constant and V is the environmental capacity, which represents the maximum density of microbes under this condition. If the thermal power given out by each microbe is P_0 then

$$P_t = P_0 N \tag{4}$$

where P_t is the thermal power at time t.

Accordingly, Eq. (5) can be obtained from Eq. (3)

$$\ln \frac{VP_0 - P_t}{P_t} = a - kt \tag{5}$$

According to exponential curve theory, the environmental capacity can be deduced [20]

$$V = \frac{2P_1P_2P_3 - P_2^2(P_1+P_3)}{P_1P_3 - P_2^2}\frac{1}{P_0} \tag{6}$$

where P_1, P_2, and P_3, are the microbial thermal powers of three equal-time-interval points.

The process of microbial growth can be divided into four phases, namely, lag phase, exponential growth phase, stationary phase and deceleration phase [21]. From the thermal power–time curve, find points at equal-time-intervals which correspond to the lag, exponential, and stationary phases of microbial growth. Using the experimental data P_t and t obtained from the thermal power–time curves under various environmental conditions, the environmental capacities V can be obtained from Eq. (6). Then the corresponding growth rate constant k can be calculated from linear regression analysis with Eq. (5).

For microbe E at 318.15 K, we have

$$\ln \frac{171.75 - P_t}{P_t} = 6.1843 - 0.02859t, \quad t \leq 350\,\text{min} \tag{7}$$

where k=0.02859, r=−0.9993.

For microbe E at 323.15 K, we have

$$\ln \frac{207.86 - P_t}{P_t} = 6.355 - 0.03063t, \quad t \leq 350\,\text{min} \tag{8}$$

where k=0.03063, r=−0.9916.

Table 1 shows the experimental data and calculated data for microbe E at 318.15 and 323.15 K in the media. From Table 1, it can be seen that the calculated P_t values are quite close to the experimental data. Similarly, the equations for thermal power–time cures of the two strains of petroleum microbe under other various environmental conditions were obtained. All the correlation coefficients are >0.99.

From the data on the microbial growth rate constant k under various environmental conditions, k–T, k–pH and k–w curves can be obtained as illustrated in Figs. 2–4. Then the optimum temperatures, acidities and salinities for the two strains can be obtained from Figs. 2–4. Table 2 presents the data. The

Table 2
Data of optimum growth conditions of petroleum microbe

Microbe	T_{opt} (K)	pH_{opt}	w_{opt} (%)
E	322.07	6.51	1.88
F	322.85	7.02	1.91

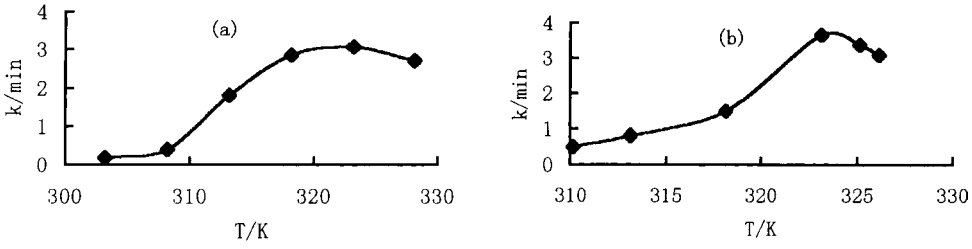

Fig. 2. *k–T* for petroleum microbes E (a) and F (b).

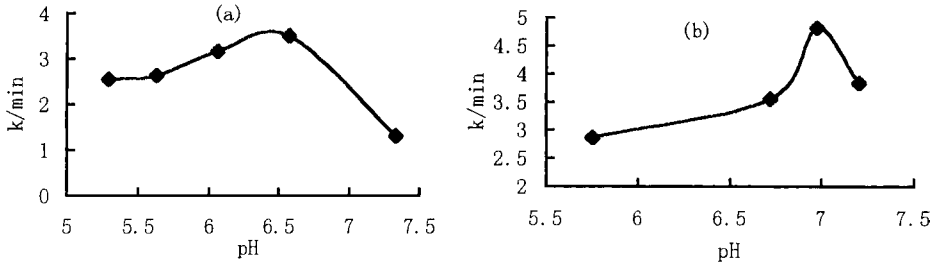

Fig. 3. *k*–pH curves for petroleum microbes E (a) and F (b).

optimum growth acidities of microbes E and F are close to neutral and they can grow at temperatures over 50°C. Microbe E grows at salinities up to 10% NaCl, while microbe F cannot. According to [20], they do not have basophilic or acidophilic growth property. They belong to the category of thermophilic microbe. The thermophilic property of microbe E is greater than that of microbe F. Microbe E belongs to the category of halophilic microbe. In conclusion, these two strains of petroleum microbe exhibit the desirable properties of rapid growth and metabolism under environmental conditions with salinity as high as 8% NaCl and temperatures as high as 53°C. Maximal metabolic activities of the two isolates are observed at 2% NaCl and around 50°C.

3.2. The growth in alkane medium

Petroleum is a complex mixture and long-chain alkanes are an important component. The thermal power–time curves for microbe E in alkane medium at 323.15 K are shown in Fig. 5. All the thermal power–time curves under the same cultivation conditions were determined twice in this work. The reproducibility was satisfactory.

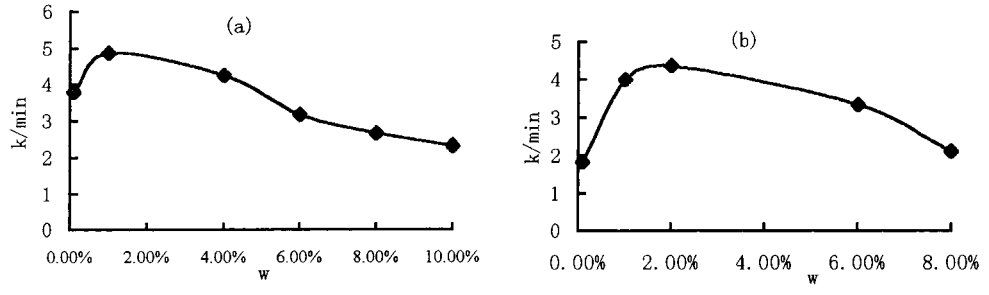

Fig. 4. *k–w* curves for petroleum microbes E (a) and F (b).

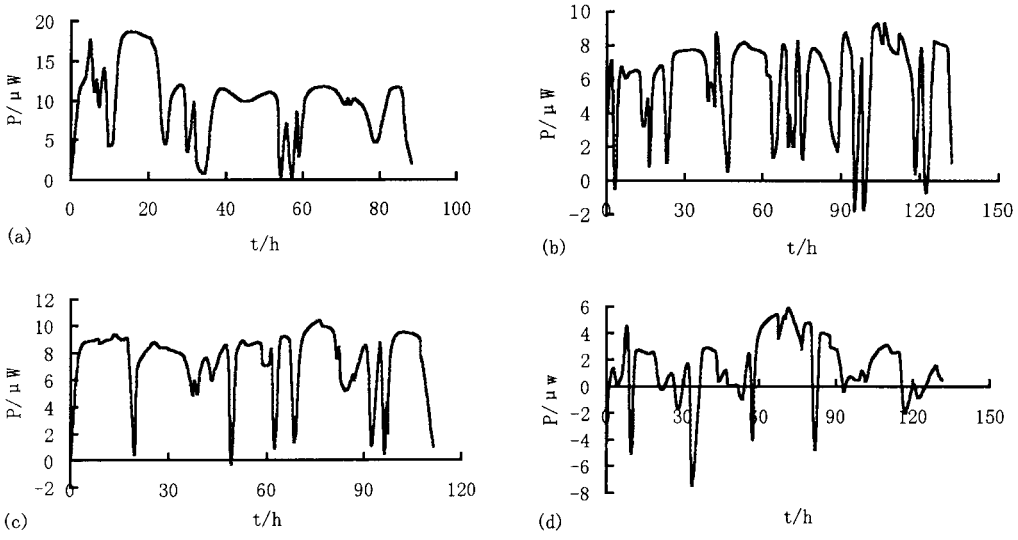

Fig. 5. Power–time curves of petroleum microbe E in the medium D (pH=7.00, w=2%) at 323.15 K: (a) *n*-dodecane; (b) *n*-tetrasdecane; (c) *n*-hexadecane and (d) *n*-octane.

Comparing Fig. 5 with Fig. 1, microbe E shows a different thermal behavior in glucose medium and in alkane medium. Firstly, the values in the thermal power in the alkane media are much lower than those in glucose. Secondly, the shape of the curve in alkane media is very irregular. In glucose medium, thermal power increases exponentially after a lag phase, and finally returns to the initial baseline because the glucose added has been totally exhausted by microbe E. In alkane medium, the irregular thermal power–time curves must be due to genuine metabolic fluctuations of the microbe under the culture conditions. Negative values of the thermal power mean that the microbial growth is inhibited at that time. Thirdly, the growth time of microbe E in alkane medium is comparatively long. A reasonable explanation is that the petroleum microbe E cuts long-chain alkanes into shorter ones to prolong its growth time.

The curves of Fig. 5 are irregular. They have no pattern or order. There is no obvious oscillating period and no regularly-varied amplitude in them. But it is very important to find that petroleum microbe E is able to exist for a considerable long time by using long-chain alkane as its initial carbon and energy source. Such microbe will play an important role in the development of enhanced oil recovery processes.

4. Conclusions

Our studies on the growth of microbes E and F isolated from the subsurface show that they have the potential for MEOR. The conditions occurring in many oil reservoirs are optimal for their growth and metabolic activities. Microbe E is the more promising object for MEOR technique because it withstands more adverse circumstances.

In this paper, the optimum growth conditions (including temperature, acidity and salinity) of two strains of petroleum microbe were determined by a microcalorimetric method. The behavior of petroleum microbe E using long-chain alkane for carbon source was observed. The research has value in practical applications and theoretical directions for studying MEOR. Subsequent investigations will elucidate the mechanism of the irregular thermal power–time curves obtained in this work.

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