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THERMO-KINETIC INVESTIGATION OF OPTIMUM GROWTH TEMPERATURE OF PETROLEUM BACTERIA

Z.-D. Nan[1], *Y. Xiang*[1], *X.-C. Zeng*[1*], *H.-L. Zhang*[2] *and H.-T. Sun*[2]

[1]Department of Chemistry, Sichuan University, Chengdu 610064, P. R. China
[2]Department of Chemistry, Qufu Normal University, Qufu 273165, P. R. China

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Abstract

The power-time curves of the growth of three strains of petroleum bacteria at different temperatures have been determined. A novel equation of a power-time curve has been proposed in this paper. The general formula to calculate the rate constant of the bacterial growth has been derived. The rate constants of the bacterial growth at different temperatures, the heat production per newly formed bacterium, the bacterial number at the end of the bacterial growth and the deceleration rate constant of the bacterial growth at 50.00°C, have been calculated. The optimum growth temperatures of the three strains have been obtained.

Keywords: microbial enhanced oil recovery, optimum growth temperature, petroleum bacteria, thermo-kinetics

Introduction

Recovering crude oil is generally divided into two periods: In the first period, crude oil spouts out from an oil well by means of the pressure of the earth; In the second period, it is by means of the pressure of the water which has been pumped into the oil well. However, there are a large number of barrels of crude oil which cannot be recovered by these two periods. So, it is of economic importance to develop recovery methods which can tap this large resource, being regarded as the third period of recovered crude oil. Microbial enhanced oil recovery (MEOR) has been attracting increasing attention [1–3] since Beckman proposed it [4] because the decomposition of petroleum by microorganisms can reduce interface tension of water-oil and oil-rock, and produce biosurfactant in oil reservoirs, and thereby be used for enhanced oil recovery. However, the bacteria selected should be able to grow under the conditions in oil reservoirs. Thus, it is important and necessary for enhanced oil recovery to study the growth conditions of petroleum bacteria.

* Author to whom all correspondence should be addressed.

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On the basis of the heat production rate proportional to the bacterial number, microcalorimetry is widely used to study the bacterial growth with the Multhus equation [5, 6] and with the logistic equation [7, 8]. Next Holzel [9] supposed that the heat production rate is proportional to the first derivative of the bacterial number with respect to time deriving the equation of a power-time curve equation with the Multhus equation. However, to our knowledge, the equation of a power-time curve for the bacterial growth with the logistic equation has not been reported. In this paper, based on the supposition by Holzel [9], a novel equation of a power-time curve for the bacterial growth is derived, by which the growth rate constants of three strains at different temperatures are calculated and the optimum growth temperature corresponding to the maximum growth rate constant is obtained. These obtained optimum growth temperatures are useful theoretically and practically for further investigations on petroleum bacteria.

Theory and method

The bacterial growth is often limited by some external conditions, including substrate or product concentrations, pH values, poisoning effects of metabolites and lack of oxygen. This situation is described by the equation of Verhulst and Pearl (logistic function) developed from the Multhus equation [9]

$$\mathrm{d}N(t)/\mathrm{d}t=kN(t)-\beta N(t)^2 \qquad (1)$$

where $N(t)$ being the number of bacteria at time t, k the growth rate constant, β the deceleration rate constant and t the experimental time.

On integrating Eq. (1) with respect to time t, we obtain

$$N(t)=N_\infty/[1+[(N_\infty-N_0)/N_0]e^{-kt}] \qquad (2)$$

where N_∞ being the bacterial number at the end of bacterial growth, N_0 the bacterial number at time zero. When $N(t)=N_\infty$, $\mathrm{d}N(t)/\mathrm{d}t=0$, we have

$$\beta=k/N_\infty \qquad (3)$$

Let

$$(N_\infty-N_0)/N_0=M \qquad (4)$$

where M is the relative factor of the increase of the bacterial number at the end of the bacterial growth.

From literature [9], we have

$$P(t)=Q_0\mathrm{d}N(t)/\mathrm{d}t \qquad (5)$$

where $P(t)$ is the heat production rate at time t, Q_0 the heat production per newly formed bacterium.

The total heat production from time 0 to t is found by integrating Eq. (5)

$$Q(t)=\int_0^t P(t)\mathrm{d}t=\int_0^t Q_0\mathrm{d}N(t)=Q_0[N(t)-N_0]=a \tag{6}$$

where a is the peak area of the power-time curve before time t. Therefore, we have

$$Q_\infty=Q_0(N_\infty-N_0)=A \tag{7}$$

where Q_∞ is the total heat produced during the bacterial growth, A the total area under the power-time curve.

From Eqs (2), (4) and (6), we obtain

$$\ln[(M\Phi+1)/(1-\Phi)]=kt \tag{8}$$

with

$$\Phi=Q(t)/Q_\infty=a/A \tag{9}$$

Equation (8) is the integrating equation of a power-time curve of bacterial growth.

Two data (Φ_1 and Φ_2) can be obtained for a fixed time interval ($\Delta t=t_2-t_1$, $t_2=2t_1$) in a power-time curve. According to Eqs (2), (4), (6) (8), it can be proved that,

$$k=(1/\Delta t)\ln[(\Phi_2-\Phi_1)/\Phi_1(1-\Phi_2)] \tag{10}$$

where $\Phi_1=a_1/A$, $\Phi_2=a_2/A$, a_1 and a_2 are the peak areas before time t_1 and t_2, respectively.

Experimental and material

Instrument

A 2277 Thermal Activity Monitor was used to determine the power-time curves of bacterial growth.

Experimental method

In the calorimetric experiment, the stopped-flow operating mode was used. The flow vessel (0.6 ml) was completely cleaned and sterilized firstly. The procedure was: sterilized distilled water, 0.1 mol L^{-1} HCl, 0.1 mol L^{-1} NaOH, 75% alcohol solution and sterilized distilled water, were pumped by a LKB-2132 microperpex peristaltic pump through the vessel.

Once the system was cleaned and sterilized, and the base line had been stabilized, the bacterial sample (initially containing $4.98\cdot10^5$ cells ml^{-1}) was pumped into the flow vessel system and a power-time curve of continuous bacterial growth was recorded. The re-establishment of a stable baseline indicated that the process of the bacterial growth was complete.

Material

Three strains of petroleum bacteria, I, II and III, employed were isolated from the oil well with depths ranging from 800 to 1200 m, temperature about 50°C.

Liquid medium (pH=7.0–7.2) containing NaCl (0.5 g), $Mg_2SO_4 \cdot 7H_2O$ (0.05 g), yeast extract (0.1 g), glucose (2.0 g) per 100 ml water was used, which was sterilized at 120°C for 30 min.

Results and discussion

The power-time curves of the bacterial growth at different temperatures were determined illustrated in Fig. 1 with 50.00°C. Experiments indicated that the power-time curve of each bacterium shows highly reproducible growth patterns under the same conditions.

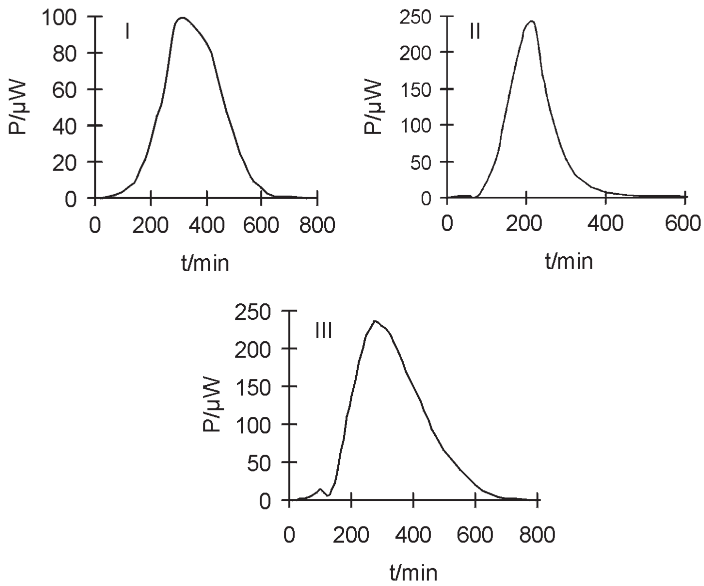

Fig. 1 Power-time curves of petroleum bacteria growth at 50°C

The data of a, A, and t are obtained from the power-time curve. Thus Φ, k, M, N_∞, Q_0 and β are calculated according to Eqs (9), (10), (8), (4), (7) and (3). A=583 μJ, k=0.0268 min^{-1}, M=9.64·10^3, N_∞=4.80·10^9 cells ml^{-1}, Q_0=0.121 pJ, β= 5.56·10^{-12} ml min^{-1} $cells^{-1}$ with I; A=788 J, k=0.0408 min^{-1}, M=2.63·10^4, N_∞= 1.31·10^{10} cells ml^{-1}, Q_0=0.0607 pJ, β=3.11·10^{-12} ml min^{-1} $cells^{-1}$ with II; A=853 μJ, k=0.0331 min^{-1}, M=1.26·10^4, N_∞=6.27·10^9 cells ml^{-1}, Q_0=0.136 pJ, β= 5.28·10^{-12} ml min^{-1} $cells^{-1}$ with III at 50.00°C. The integrating equations of power-time curves are derived and illustrated below with 50.00°C.

For I

$$\ln(9.64 \cdot 10^{3}\,\Phi+1)/(1-\Phi)=0.0268t \tag{11}$$

For II

$$\ln(2.63 \cdot 10^{4}\,\Phi+1)/(1-\Phi)=0.0408t \tag{12}$$

For III

$$\ln(1.26 \cdot 10^{4}\,\Phi+1)/(1-\Phi)=0.0331t \tag{13}$$

The rate constants of the bacterial growth at different temperatures are obtained with the same method. The graphs of the rate constants of the bacterial growth with respect to temperatures are shown in Fig. 2. The form of a non-linear equation as $k=a+bT+cT^2+dT^3$ can be established from these results. These render the foreseeing optimum growth temperatures T_m. For I, T_m=51.39°C; for II, T_m=50.99°C; for III, T_m=50.55°C.

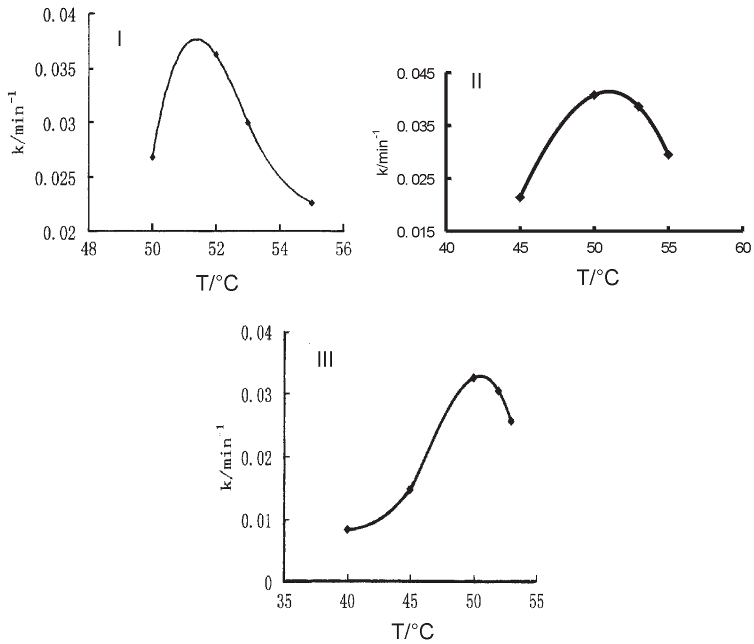

Fig. 2 Plots of bacterial growth constants *k vs.* bacterial growth temperatures *T*

In literature [10], four strains isolated from Northern German oil reservoirs at depth of 866–1520 m grew at temperatures up to 55°C. I and II grew at temperatures up to 55°C. III grew at temperature up to 53°C. The optimum growth temperatures of I, II and III are 51.39, 50.99 and 50.55°C, respectively. A decomposition of petroleum by microorganisms can be studied at the optimum growth temperature.

From the data of A, M, k, N_∞, Q_0 and β above, it can be seen that the greater the data of k are, the greater the data of M and N_∞ are, and the smaller the data of β are, the data of A has relationship with Q_0 and k. The equation of a power-time curve presented in this paper describes the relationship between the production heat of the bacterial growth and the bacterial number. Thus, the total bacterial number N_∞ contains the total bacteria in the system because per newly formed bacterium can produce heat. In literatures [5–8], the live bacteria can only be studied because death bacteria cannot produce heat production rate at any time.

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