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Thermokinetic investigation of effect of temperature on optimum NaCl concentration for petroleum bacterial growth

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Abstract

The power–time curves of growth of three strains of petroleum bacteria at different NaCl concentrations at 40.0 and 50.0 °C have been determined by using a 2277 Thermometric Thermal Activity Analyser. An equation of a power–time curve, $\ln[\alpha P_K/P(t) - 1] = \ln[(\alpha K - N_0)/N_0] - \alpha kt$, was established based on the generalized logistic equation, where $P(t)$ is the thermal power at time t, K the carrying capacity, $P_K = P_0K$, P_0 the thermal power of one cell, N_0 the bacterial population at time zero, $\alpha = (k - D)/k$. The method of four observed points with the same time interval was used to calculate the value of P_K. The growth rate constant k and the death rate constant D were calculated. The NaCl concentration of optimum growth rate of petroleum bacteria at 40.0 and 50.0 °C, respectively, have been obtained according to the curves $k - D$ versus NaCl concentration, which are 0.26, 0.54 and 0.57 mol l^{-1} for B-1, B-2 and B-3, respectively, at 50.0 °C, 0.26, 0.55 and 0.56 mol l^{-1} for B-1, B-2 and B-3, respectively, at 40.0 °C. The results indicated that the effect of temperature on NaCl concentration of optimum growth rate was small. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Thermokinetics; Petroleum bacteria; Microbial enhanced oil recovery; Temperature; NaCl concentration

1. Introduction

The growth conditions of microorganisms isolated from oil reservoirs to increase oil recovery have been extensively investigated [1–3]. In the previous papers [4,5], we have reported on the investigation of the optimum conditions of petroleum bacterial growth and the effect of bacterial growth temperature on the optimum acidity by using of microcalorimetric method. The temperature is different at different depth in oil reservoirs. Therefore, it is of interest to study the effects of temperature on NaCl concentration of bacterial optimum growth.

The simplest growth model of bacteria is Malthus equation. The bacterial growth is often limited by some external conditions, including substrate or product concentrations, pH values, poisoning effect of metabolites and lack of oxygen. This situation is described by the equation of Verhulst and Pearl (logistic function) developed from the Malthus equation [6]. Some bacteria may die during bacterial growth. Therefore, the generalized logistic equation is developed from logistic equation [7].

In this paper, a novel equation of a power–time curve is derived based on the generalized logistic equation. The growth and death rate constants of strains at different NaCl concentrations at 40.0 and

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50.0 °C are calculated. The NaCl concentrations of optimum growth of three strains are obtained at 40.0 and 50.0 °C, respectively. The results indicate that the effect of temperature on NaCl concentration of optimum growth is small.

2. Theory and methods

2.1. Establishment of an equation for a power–time curve

The generalized logistic equation is described as [7]

$$\frac{dN(t)}{dt} = kN(t)\left[\frac{1-N(t)}{K}\right] - DN(t) \tag{1}$$

where $N(t)$ is the bacterial population at time t, k the growth rate constant, D the death rate constant, K the carrying capacity and t the experimental time. Carrying capacity K is defined as theoretical value of the maximum bacterial population at the experimental conditions.

When $D = 0$, we have

$$\frac{dN(t)}{dt} = kN(t)\left[\frac{1-N(t)}{K}\right] \tag{2}$$

Eq. (2) is the logistic equation. Thus, Eq. (1) is more suitable for bacterial growth of separated culture.

From literature [8], we have

$$P(t) = P_0N(t) \tag{3}$$

where $P(t)$ is the thermal power at time t, P_0 the thermal power of one cell.

Inserting Eq. (3) into Eq. (1), we have

$$\frac{dP(t)}{dt} = kP(t)\left[\frac{1-P(t)}{KP_0}\right] - DP(t) \tag{4}$$

Let

$$P_K = P_0K \tag{5}$$

From Eqs. (4) and (5), we obtain

$$\frac{dP(t)}{dt} = kP(t)\left[\frac{1-P(t)}{P_K}\right] - DP(t) \tag{6}$$

Eq. (6) is the differential equation of a power–time curve.

On integrating Eq. (1) with respect to t, we prove that

$$\ln\left[\frac{(k-D)P_K/k}{P(t)-1}\right] = \ln\left[\frac{((k-D)(K-N_0)/k)}{N_0}\right] - (k-D)t \tag{7}$$

where N_0 is the bacterial population at time zero.

We define a factor α

$$\alpha = \frac{k-D}{k} \tag{8}$$

We have

$$\ln\left[\frac{\alpha P_K}{P(t)-1}\right] = \ln\left[\frac{\alpha K - N_0}{N_0}\right] - \alpha kt \tag{9}$$

When $\alpha > 0$, $k > D$, bacteria are in growth phase, and bacterial population is increasing as experimental time increases. When $\alpha = 0$, $k = D$, bacterial population stays constant. When $\alpha < 0, k < D$, bacterial population is decreasing as experimental time increases. Eq. (9) is suitable for $\alpha > 0$. Because $D > 0$, we have $1 > \alpha > 0$.

Eq. (9) is the equation of a power–time curve of bacterial growth.

From Eq. (9), we have $P_m < \alpha P_K < P_K$ with P_m being the maximum heat production rate during bacterial growth. The value of α can be obtained by obtaining the best coefficient relativity from linear regression analysis with Eq. (9) between 1 and 0.

2.2. Determination of value of P_K

A group of four observed points with a same time interval is used for calculating a value of P_K.

From literature [9], we have

$$K = \frac{N_2N_3(N_1+N_4) - N_1N_4(N_2+N_3)}{N_2N_3 - N_1N_4} \tag{10}$$

where N_1, N_2, N_3 and N_4 are the populations of bacteria at time t_1, t_2, t_3 and t_4, respectively, and $t_2 - t_1 = t_3 - t_2 = t_4 - t_3$.

Inserting Eq. (10) into Eq. (5), we have

$$P_K = \frac{P_2P_3(P_1+P_4) - P_1P_4(P_2+P_3)}{P_2P_3 - P_1P_4} \tag{11}$$

where P_1, P_2, P_3 and P_4 are the thermal power at time t_1, t_2, t_3 and t_4, respectively.

Thus, many values of P_K are obtained with all of such four points and the mean value of these values of P_K is used as the value of P_K in Eq. (9).

2.3. Determination of NaCl concentration of optimum growth

The values of k, D and $k - D$ are calculated from linear regression analysis with Eq. (9). The curves of $k - D$ versus NaCl concentration are obtained. The maximum in the data of $k - D$ leads to the NaCl concentration of optimum growth.

3. Experimental

3.1. Instrument

A 2277 Thermal Activity Monitor (Thermometric AB, Sweden) was used to determine the thermal power–time curves of bacterial growth. With this instrument, reactions can be studied in the temperature range 10–80 °C (the working temperature range of the thermostat). It was maintained at a temperature within $\pm 2 \times 10^{-4}$ K. The detection limit was 0.15 μW and the baseline stability (over a period of 24 h) was

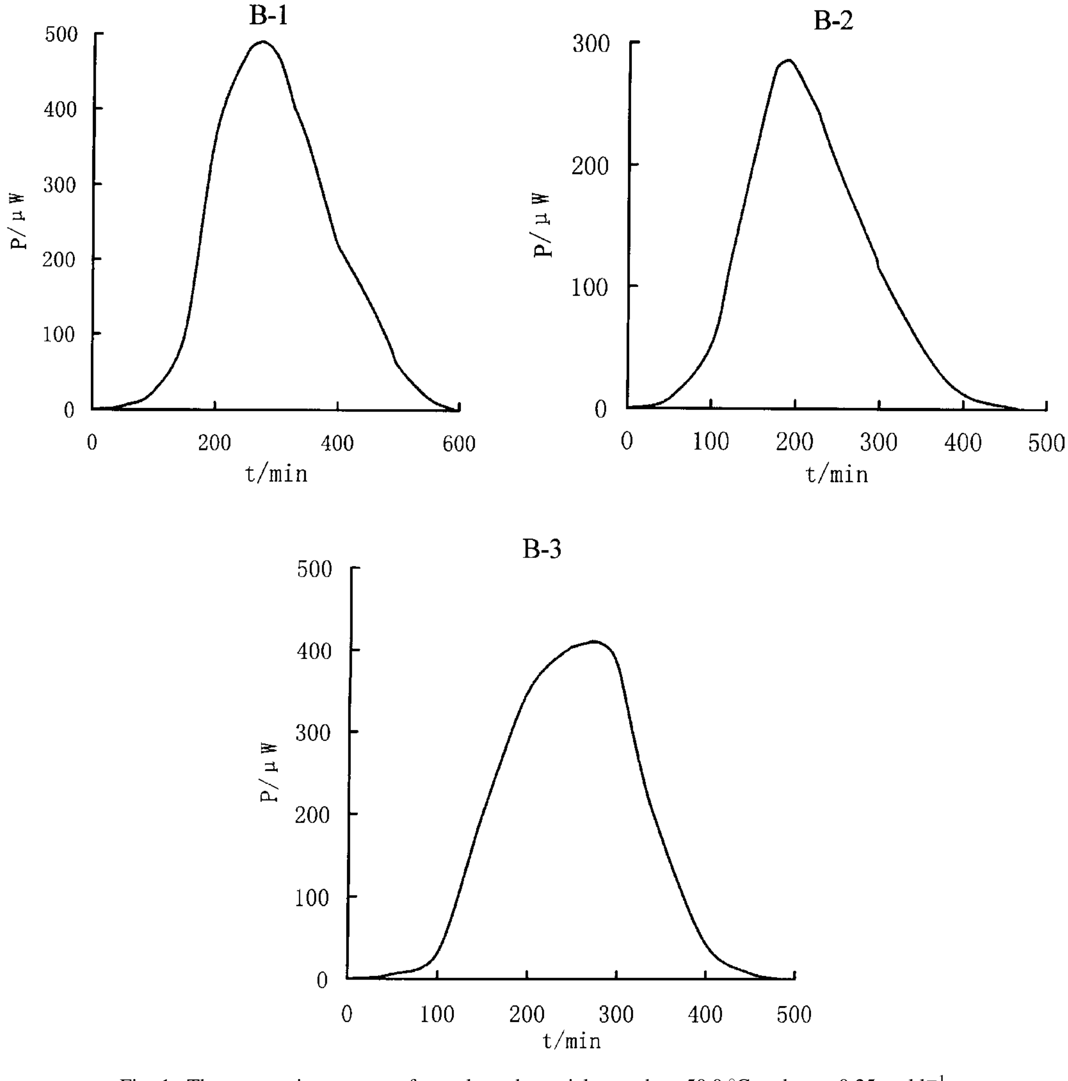

Fig. 1. The power–time curves of petroleum bacterial growth at 50.0 °C and $m = 0.25$ mol l^{-1}.

0.2 μW. The performance of this instrument and the details of its construction have been previously described [10].

3.2. Experimental method

In the calorimetric experiments, the stopped-flow operating mode was used and the sample was pumped through the flow vessel by a Micro Perpex pump (LKB2132, Sweden).

The consecutive steps of complete cleaning and sterilization for the flow tubing were as follows:

- Sterilized distilled water, alcohol solution (75%), 0.1 M NaOH, 0.1 M HCl and sterilized distilled water were pumped through the system for 30 min at a flow rate of 30 ml h^{-1}, respectively.
- Once the system was cleaned and sterilized, sterilized distilled water was pumped through the system at a flow rate of 10 ml h^{-1} and the baseline was obtained.
- The bacterial sample initially containing 4.98×10^5 cells ml^{-1} was pumped into the flow vessel at a flow rate of 10 ml h^{-1} when the stable baseline was obtained.
- When the flow vessel (the volume was about 0.6 ml) was full of the bacterial suspension, the pump was stopped and the power–time curve of continuous bacterial growth was recorded by the calorimeter.
- When the signal pen returned to the baseline, the process of bacterial growth was completed.

3.3. Materials

The strains of petroleum bacteria, B-1, B-2 and B-3 employed, were isolated from the oil well through enrichment culture. Petroleum was the sole carbon source in the enrichment medium.

Growth medium containing $NaNO_3$ (0.2 g), $MgSO_4 \cdot 7H_2O$ (0.05 g), $(NH_4)_2SO_4$ (0.1 g), KH_2PO_4 (0.5 g),

Table 1
The data of α, $k - D$ and D of growth of three strains of petroleum bacteria at different NaCl concentrations and temperatures

Bacteria	Temperature (°C)		Concentration (mol l^{-1})						
			0.083	0.17	0.25	0.34	0.60	0.68	1.0
B-1	40.0	α	0.6093	0.6765	0.7009	0.6908	0.5384		
		$(k - D) \times 10^2\ min^{-1}$	1.609	1.937	2.047	1.984	1.318		
		$k \times 10^2\ min^{-1}$	2.640	2.863	2.920	2.874	2.448		
		$D \times 10^2\ min^{-1}$	1.031	0.926	0.874	0.890	1.130		
	50.0	α	0.8573	0.9167	0.9416	0.9271	0.8145		
		$(k - D) \times 10^2\ min^{-1}$	4.271	4.619	4.785	4.681	3.512		
		$k \times 10^2\ min^{-1}$	4.982	5.039	5.082	5.049	4.312		
		$D \times 10^3\ min^{-1}$	7.16	4.20	2.97	3.68	8.00		
B-2	40.0	α		0.6893	0.7217	0.7408		0.7564	0.5915
		$(k - D) \times 10^2\ min^{-1}$		2.714	2.916	3.102		3.195	2.017
		$k \times 10^2\ min^{-1}$		3.937	4.040	4.187		4.224	3.410
		$D \times 10^2\ min^{-1}$		1.223	1.124	1.085		1.029	1.393
	50.0	α		0.8846	0.9181	0.9428		0.9609	0.8100
		$(k - D) \times 10^2\ min^{-1}$		4.906	5.201	5.408		5.531	4.096
		$k \times 10^2\ min^{-1}$		5.545	5.665	5.736		5.756	5.057
		$D \times 10^3\ min^{-1}$		6.39	4.64	3.28		2.25	9.61
B-3	40.0	α		0.6016	0.6375	0.6492		0.6719	0.5535
		$(k - D) \times 10^2\ min^{-1}$		1.759	1.982	2.061		2.204	1.593
		$k \times 10^2\ min^{-1}$		2.924	3.109	3.175		3.280	2.878
		$D \times 10^2\ min^{-1}$		1.164	1.127	1.114		1.076	1.285
	50.0	α		0.8594	0.8852	0.9017		0.9178	0.8014
		$(k - D) \times 10^2\ min^{-1}$		4.637	4.802	4.973		5.104	4.163
		$k \times 10^2\ min^{-1}$		5.396	5.425	5.515		5.561	5.195
		$D \times 10^3\ min^{-1}$		7.59	6.23	5.42		4.57	10.32

K_2HPO_4 (1.0 g), yeast extract (0.1 g), glucose (2.0 g), and NaCl in different amounts per 100 ml water (pH = 7.0) was used in the calorimetric experiments.

4. Results and discussion

4.1. Determination of power–time curves

The power–time curves of growth of three strains growth at different NaCl concentrations at 40.0 and 50.0 °C, respectively, have been determined. The power–time curves show highly reproducible growth patterns under the same conditions. The power–time curves of growth of these three strains of petroleum bacteria at $m = 0.25$ mol l^{-1} and 50.0 °C are shown in Fig. 1, where m is expressed as the NaCl concentration.

4.2. Establishment of an equation of a power–time curve

From the power–time curves in Fig. 1, many groups of four observed points P_1, P_2, P_3 and P_4 with a same time interval are obtained. The values of P_K are calculated according to Eq. (11), which are 534.3, 314.5 and 482.7 μW for B-1, B-2 and B-3, respectively. So, the values of α are obtained by obtaining the best coefficient relativity of linear regression analysis with Eq. (9) between 0 and 1, which are 0.9416,

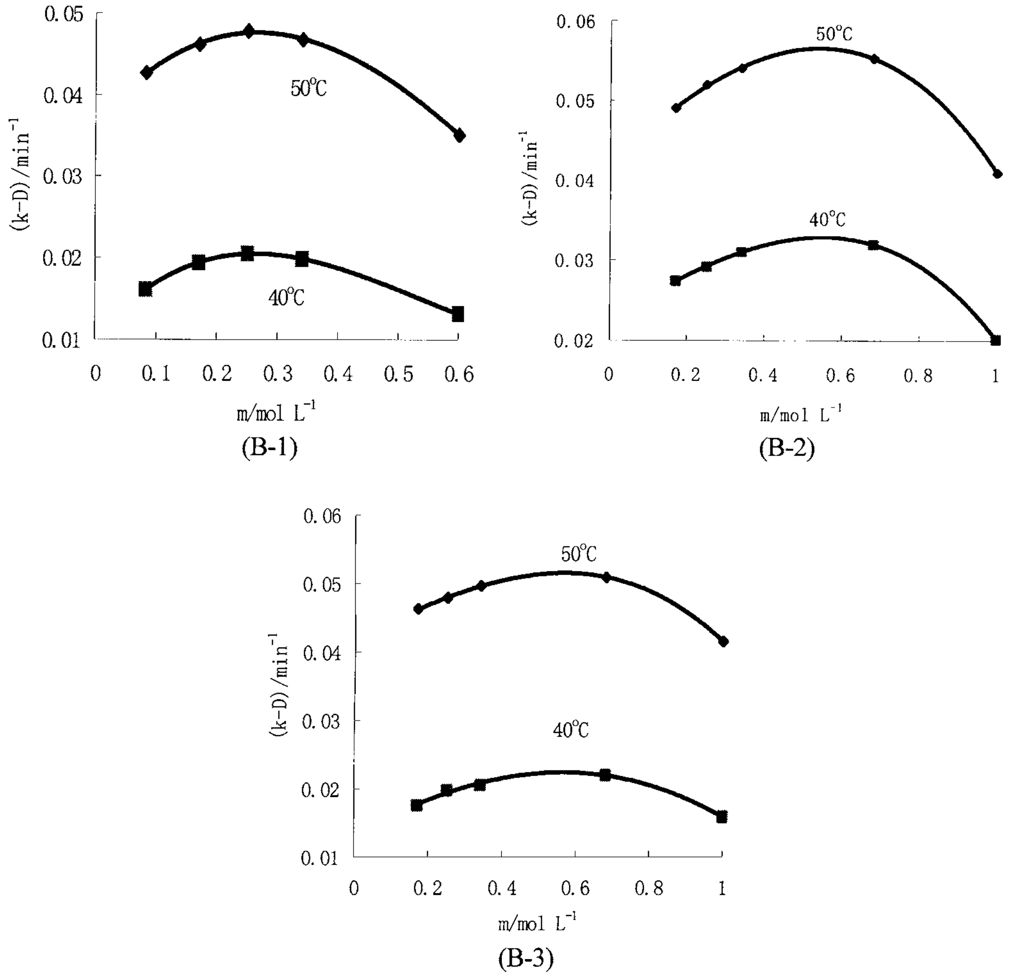

Fig. 2. Curves of $k - D$ vs. m for petroleum bacterial growth.

0.9181 and 0.8852 for B-1, B-2 and B-3, respectively. The equations of the power–time curves of petroleum bacterial growth at 50.0 °C and $m = 0.25$ mol l^{-1} are established according to Eq. (9). For B-1, we have

$$\ln\left[\frac{503.1}{P(t)-1}\right] = 8.449 - 0.04785t \quad (12)$$

where $r = -0.9998$, $k - D = 0.04785$ min^{-1}, $k = 0.05082$ min^{-1}, $D = 0.00297$ min^{-1} with r being the coefficient of relativity.

For B-2, we have

$$\ln\left[\frac{288.7}{P(t)-1}\right] = 6.641 - 0.05201t \quad (13)$$

here $r = -0.9991$, $k - D = 0.05201$ min^{-1}, $k = 0.05665$ min^{-1}, $D = 0.00464$ min^{-1}.

For B-3, we have

$$\ln\left[\frac{422.3}{P(t)-1}\right] = 7.004 - 0.04802t \quad (14)$$

where $r = -0.9999$, $k - D = 0.04802$ min^{-1}, $k = 0.05425$ min^{-1}, $D = 0.00605$ min^{-1}.

4.3. Determination of the optimum NaCl concentration for the petroleum bacterial growth

The data of α, k, D and $k - D$ of petroleum bacterial growth at different NaCl concentrations at 40.0 and 50.0 °C, respectively, are obtained to the same method as above and shown in Table 1.

The curves of $k - D$ versus m at 40.0 and 50.0 °C are obtained according to the data in Table 1 and given in Fig. 2. The maximum in $k - D$ leads to the m_m of the optimum NaCl concentration of petroleum bacterial growth. Then, the values of m_m have been obtained, which are 0.26, 0.54 and 0.57 mol l^{-1} for B-1, B-2 and B-3, respectively, at 50.0 °C, 0.26, 0.55 and 0.56 mol l^{-1} for B-1, B-2 and B-3, respectively, at 40.0 °C. From the values of m_m obtained in this paper, we can conclude that the effect of temperature on NaCl concentration of optimum growth rate of these three strains of petroleum bacteria is small. From the data of Table 1, we can conclude that the greater the value of the growth rate constant, the smaller the value of the bacterial death rate constant, the greater the values of the $k - D$ and α. Therefore, the factor α defined in this paper has the relationship with the experimental conditions of the bacterial growth.

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