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Microcalorimetric investigation of effects of temperature on pH of petroleum bacterial optimum growth

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Abstract

The power-time curves for growth at different pH values of three strains of petroleum bacteria (B-1, B-2, B-3) at 40 and 50°C have been determined by using a 2277 Thermal Activity Monitor, and on the basis of the generalized logistic equation, a power–time curve equation, $\ln[\alpha P_K/P(t)-1]=\ln[(\alpha K-N_0)/N_0]-\alpha kt$, was proposed for calculating the growth and the death rate constants k, D, and nonlinear equations of k-pH, D-pH and $(k-D)$-pH were established for deciding the pH of optimum growth corresponding to the maximum values of k, $(k-D)$ and the minimum value of D. The results indicated that the effect of temperature on pH of optimum growth was small.

Keywords: Petroleum bacteria; Microcalorimetry; Microbial enhanced oil recovery; Temperature; pH of optimum growth

1. Introduction

It is well known that microbial enhanced oil recovery (MEOR) is of economic importance compared to the conventional techniques. Thus, it is significant to find appropriate bacteria that can grow under reservoir conditions of temperature, salinity and acidity etc. In the previous paper [1], we have reported on the investigation of the optimum conditions of petroleum bacterial growth. But the temperature is different at different depths in oil reservoirs. Consequently, it is of interest to study the effect of temperature on pH of bacterial optimum growth.

In this paper, the effects of temperature on pH for optimum growth rate of three strains of petroleum bacteria were investigated by microcalorimetry, and on the basis of the generalized logistic equation, a novel power–time curve equation is derived for calculating the growth rate constants and the death rate constants of different pHs at 40.0 and 50.0°C, and nonlinear equations of k-pH, D-pH and $(k-D)$-pH were established for deciding the pH of optimum growth corresponding to the maximum values of k, $(k-D)$ and the minimum value of D. The results indicated that the effect of temperature on pH of optimum growth was small.

2. Theory and method

2.1. Establishment of a power–time curve equation for bacterial growth

Generally, it is well known that Malthus equation [2] is the simplest growth model of bacteria. After-

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wards, Verhulst and Pearl [3] developed the logistic equation based on Malthus equation since bacterial growth is often limited by some external conditions, including substrate or product concentrations, pH values, poisoning effect of metabolites and lack of oxygen. Considering that some of bacteria may be dead during bacterial growth, we propose that the generalized logistic equation be written as

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

where $N(t)$ is the bacterial population at time t, k the growth rate constant, D the death rate constant, t the time, and K the carrying capacity that is defined as theoretical value of the maximum bacterial population at the experimental condition [3].

When D=0, we have

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

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

From literature [4], we have

$$P(t) = P_0 N(t) \quad (3)$$

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

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

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

Let

$$P_K = P_0 K \quad (5)$$

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

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

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

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

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

where N_0 is the bacterial population at t=0.

We define a dimensionless parameter α:

$$\alpha = \frac{k-D}{k} \quad (8)$$

We have

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

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

2.2. Mathematical model for calculating the value of P_K: four points method

If four data (N_1, N_2, N_3, N_4) are taken at a fixed time interval, i.e. $t_2-t_1=t_3-t_2=t_4-t_3$, according to literature [5], we have

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

where N_1, N_2, N_3, N_4 are the populations of bacteria at times t_1, t_2, t_3, t_4, respectively.

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

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

where P_1, P_2, P_3, P_4 are the thermal power from the same power–time curve at times t_1, t_2, t_3, t_4, respectively.

Eq. (11) is called the mathematical model for calculating the value of P_K.

3. Experimental

3.1. Instruments

A 2277 Thermal Activity Monitor (ThermoMetric AB, Sweden) was used to determine the power–time curves of bacterial growth. With this instrument, reactions can be carried out in the temperature range 10–80°C (the working temperature range of the thermostat). It was maintained at a temperature within $\pm 2\times10^{-4}$ K. The detection limit was 0.15 μW, and the baseline stability (over a period of 24 h) was 0.2 μW. The performance of this instrument and the details of its construction have been described previously [6].

A glass electrode pH-meter was used (mode HM-205, TOA Electronics, Japan) with a range of pH= 0.00–14.00.

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, respectively. Once the system was cleaned and sterilized, sterilized distilled water was pumped through the system at a flow rate of 10 ml/h and the baseline was obtained. The bacterial sample initially containing 4.98×10^5 cells/ml was pumped into the flow vessel at a flow rate of 10 ml/h, following which 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 calorimetry recorded the power–time curve of continuous bacterial growth. When the signal pen

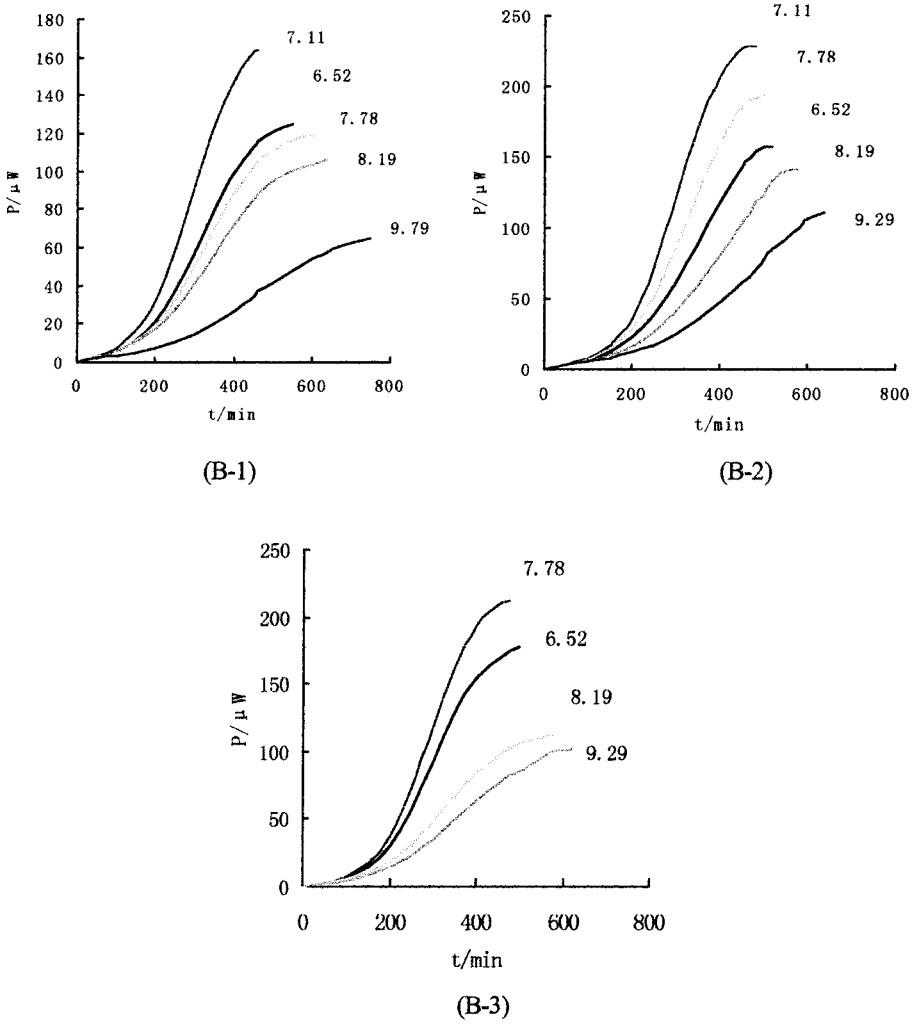


Fig. 1. The power–time curves of petroleum bacterial growth at 50.0°C and different pHs.

returned to the baseline, the process of bacterial growth was completed.

3.3. Material

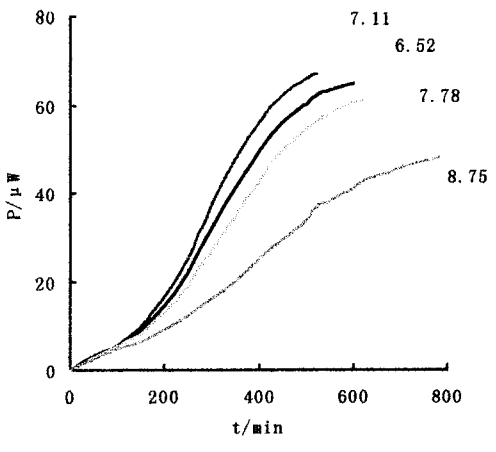
The strains of petroleum bacteria, B-1, B-2, B-3, employed were isolated from the oil well through enrichment culture. Petroleum was the sole carbon source in the enrichment medium.

The growth medium used in the calorimetric experiments was NaCl (0.5 g), $NaNO_3$ (0.2 g), $MgSO_4 \cdot 7H_2O$ (0.05 g), $(NH_4)_2SO_4$ (0.1 g), KH_2PO_4 (0.5 g), K_2HPO_4 (1.0 g), yeast extract (0.1 g), glucose (2.0 g), per 100 ml buffer solution.

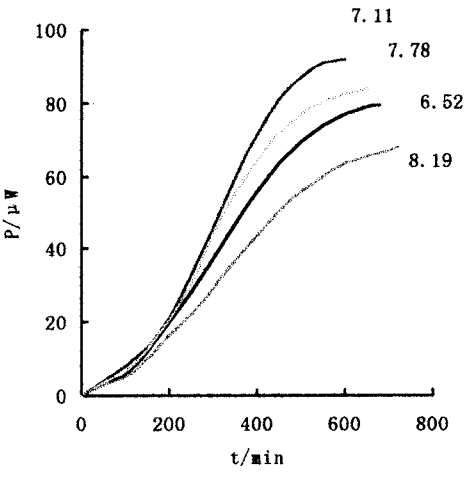
The buffer solution consisted of H_3PO_4 (3.92 g, 85 vol.%), CH_3COOH (2.40 g), H_3BO_3 (2.47 g) and NaOH in different amounts per 1000 ml water.

4. Results and discussion

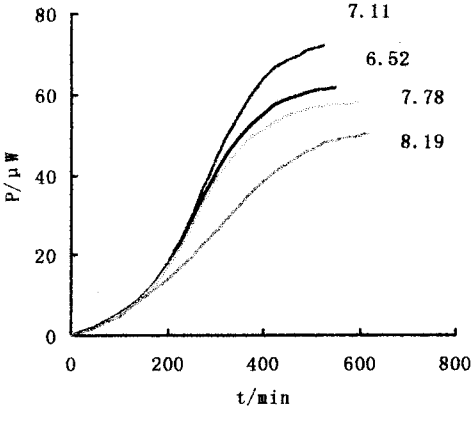
From Eq. (8), it can be seen that, when $\alpha > 0$, $k > D$, bacteria are growing and the bacterial population is increasing as the experiment is proceeding; when



(B-1)



(B-2)



(B-3)

Fig. 2. The power–time curves of petroleum bacterial growth at 40.0°C and different pHs.

Table 1
Equations of power–time curves at 50.0°C

Bacteria	pH	Equations of power–time curves	α	K (cells/ml)	r
B-1	6.52	$\ln[129.6/P(t)-1]=4.436-0.01407t$	0.8725	4.88×10^7	0.9974
	7.11	$\ln[175.8/P(t)-1]=4.711-0.01523t$	0.9136	6.11×10^7	0.9992
	7.78	$\ln[122.8/P(t)-1]=4.297-0.01316t$	0.8471	4.38×10^7	0.9895
	8.19	$\ln[109.5/P(t)-1]=4.025-0.01173t$	0.7926	3.58×10^7	0.9902
	9.79	$\ln[70.2/P(t)-1]=3.819-0.008375t$	0.7091	3.27×10^7	0.9847
B-2	6.52	$\ln[187.0/P(t)-1]=4.503-0.01256t$	0.8436	5.39×10^7	0.9943
	7.11	$\ln[244.8/P(t)-1]=5.194-0.01726t$	0.9328	9.67×10^7	0.9976
	7.78	$\ln[217.6/P(t)-1]=4.862-0.01470t$	0.8931	7.26×10^7	0.9962
	8.19	$\ln[172.4/P(t)-1]=4.374-0.01062t$	0.8193	4.88×10^7	0.9925
	9.79	$\ln[146.2/P(t)-1]=4.087-0.008362t$	0.7587	3.98×10^7	0.9939
B-3	6.52	$\ln[185.4/P(t)-1]=4.845-0.01614t$	0.9146	6.98×10^7	0.9943
	7.78	$\ln[225.2/P(t)-1]=5.021-0.01704t$	0.9427	8.06×10^7	0.9986
	8.19	$\ln[117.0/P(t)-1]=4.293-0.01302t$	0.8207	4.50×10^7	0.9907
	9.29	$\ln[109.8/P(t)-1]=4.017-0.01083t$	0.7815	3.60×10^7	0.9853

$\alpha=0$, $k=D$, the bacterial population remains constant; when $\alpha<0$, $k<D$, the bacterial population is decreasing as the experiment is proceeding. In addition, because $D>0$, $\alpha<1$, from Eq. (9), it can be noted that Eq. (9) is only suitable for $\alpha>0$. As a result, from all the above, we have $1>\alpha>0$.

The power–time curves of different pH values for the growth of three strains at 40.0 and 50.0°C have been determined by a microcalorimeter. The power–time curves show highly reproducible growth patterns under the same conditions. Typical graphs at growth phase (i.e. $\alpha>0$) are shown in Figs. 1 and 2.

According to Eq. (11), we have obtained a value of P_K which is the mean value of at least five determinations from Figs. 1 and 2. From Eq. (9), it can be seen that $P_m<\alpha P_K<P_K$ with P_m being the maximum heat production rate during bacterial growth. Thus, the value of α between 1 and 0 can be obtained from linear regression analysis with the best relative coefficient with respect to Eq. (9). Consequently, the power–time curve equations of petroleum bacterial growth are obtained and are shown in Tables 1 and 2.

According to the established power–time curve equations in Tables 1 and 2, we can obtain the growth and the death rate constants k, D and $(k-D)$ of different pHs at 40.0 and 50.0°C by comparison with Eqs. (8) and (9). The results are shown in Table 3.

Table 2
Equations of power–time curves at 40.0°C

Bacteria	pH	Equations of power–time curves	α	K (cells/ml)	r
B-1	6.52	$\ln[67.2/P(t)-1]=3.583-0.01152t$	0.8316	2.21×10^7	0.9941
	7.11	$\ln[70.6/P(t)-1]=3.746-0.01275t$	0.8854	2.44×10^7	0.9976
	7.78	$\ln[64.5/P(t)-1]=3.425-0.01024t$	0.8072	1.96×10^7	0.9825
	8.75	$\ln[50.8/P(t)-1]=3.017-0.007461t$	0.7235	1.48×10^7	0.9953
B-2	6.52	$\ln[82.5/P(t)-1]=2.985-0.009316t$	0.8103	1.28×10^7	0.9863
	7.11	$\ln[96.4/P(t)-1]=3.572-0.01158t$	0.8901	2.05×10^7	0.9923
	7.78	$\ln[86.0/P(t)-1]=3.089-0.01050t$	0.8454	1.35×10^7	0.9974
	8.19	$\ln[70.5/P(t)-1]=2.875-0.008425t$	0.7416	1.26×10^7	0.9892
B-3	6.52	$\ln[62.8/P(t)-1]=3.508-0.01375t$	0.8588	1.99×10^7	0.9893
	7.11	$\ln[74.2/P(t)-1]=4.025-0.01463t$	0.8734	3.25×10^7	0.9947
	7.78	$\ln[58.5/P(t)-1]=3.274-0.01314t$	0.8264	1.65×10^7	0.9927
	8.19	$\ln[52.5/P(t)-1]=3.014-0.01003t$	0.7019	1.52×10^7	0.9857

Table 3
Data of k, D and $(k-D)$[a]

T (°C)	Bacteria	pH	k (min^{-1})	D (min^{-1})	$(k-D)$ (min^{-1})
50.0	B-1	6.52	0.01613	0.00206	0.01407
			(0.01615)	(0.00205)	(0.01403)
		7.11	0.01667	0.00144	0.01523
			(0.01659)	(0.00149)	(0.01533)
		7.78	0.01554	0.00238	0.01316
			(0.01568)	(0.00230)	(0.01348)
		8.19	0.01480	0.00307	0.01173
			(0.01472)	(0.00311)	(0.01154)
		9.79	0.01181	0.00344	0.008375
			(0.01181)	(0.00344)	(0.008383)
50.0	B-2	6.52	0.01489	0.00233	0.01256
			(0.01485)	(0.00232)	(0.01253)
		7.11	0.01850	0.00124	0.01726
			(0.01864)	(0.00127)	(0.01737)
		7.78	0.01646	0.00176	0.01470
			(0.01622)	(0.00171)	(0.01452)
		8.19	0.01312	0.00250	0.01062
			(0.01310)	(0.00237)	(0.01073)
		9.79	0.01102	0.00266	0.008362
			(0.01101)	(0.00266)	(0.008357)
50.0	B-3	6.52	0.01765	0.00151	0.01614
			(0.01765)	(0.00151)	(0.01614)
		7.78	0.01808	0.00104	0.01704
			(0.01808)	(0.00104)	(0.01704)
		8.19	0.01586	0.00284	0.01302
			(0.01586)	(0.00284)	(0.01302)
		9.29	0.01386	0.00303	0.01083
			(0.01386)	(0.00303)	(0.01083)
40.0	B-1	6.52	0.01385	0.00233	0.01152
			(0.01385)	(0.00233)	(0.01152)
		7.11	0.01440	0.00165	0.01275
			(0.01440)	(0.00165)	(0.01275)
		7.78	0.01269	0.00245	0.01024
			(0.01269)	(0.00245)	(0.01024)
		8.75	0.01013	0.00285	0.007461
			(0.01013)	(0.00285)	(0.007461)
40.0	B-2	6.52	0.01150	0.00218	0.009316
			(0.01150)	(0.00218)	(0.009316)
		7.11	0.01301	0.00143	0.01158
			(0.01301)	(0.00143)	(0.01158)
		7.78	0.01242	0.00192	0.01050
			(0.01242)	(0.00192)	(0.01050)
		8.19	0.01136	0.00294	0.008425
			(0.01136)	(0.00294)	(0.008425)
40.0	B-3	6.52	0.01601	0.00226	0.01375
			(0.01601)	(0.00226)	(0.01375)
		7.11	0.01675	0.00212	0.01463
			(0.01675)	(0.00212)	(0.01463)
		7.78	0.01590	0.00276	0.01314
			(0.01590)	(0.00276)	(0.01314)
		8.19	0.01429	0.00426	0.01003
			(0.01429)	(0.00426)	(0.01003)

[a] Values in parentheses calculated from corresponding equations in Table 4.

Table 4
Non-linear equations of k-pH, D-pH and $(k-D)$-pH

T (°C)	Bacteria	Non-linear equation	pH_m
50.0	B-1	$k=-0.224209+0.0904284pH-0.0110882pH^2+4.40651\times10^{-4}pH^3$	6.99
		$k-D=-0.606946+0.236081pH-0.0293840pH^2+1.19403\times10^{-3}pH^3$	7.03
		$D=0.303642-0.115558pH+0.0145320pH^2-5.98627\times10^{-4}pH^3$	7.03
50.0	B-2	$k=-1.31824+0.504506pH-0.0626785pH^2+2.55511\times10^{-3}pH^3$	7.15
		$k-D=-1.6402+0.625019pH-0.0776016pH^2+3.16239\times10^{-3}pH^3$	7.19
		$D=0.321932-0.120489pH+0.0149199pH^2-6.07124\times10^{-4}pH^3$	7.22
50.0	B-3	$k=-1.31336+0.509990pH-0.0643056pH^2+2.66817\times10^{-3}pH^3$	7.12
		$k-D=-2.02885+0.786015pH-0.0994555pH^2+4.14214\times10^{-3}pH^3$	7.11
		$D=0.715490-0.276025pH+0.0351500pH^2-1.47396\times10^{-3}pH^3$	7.07
40.0	B-1	$k=-0.577407+0.231725pH-0.0298934pH^2+1.26707\times10^{-3}pH^3$	6.92
		$k-D=-1.0520+0.418151pH-0.0542181pH^2+2.31622\times10^{-3}pH^3$	6.96
		$D=0.474463-0.186383pH+0.0243187pH^2-1.04887\times10^{-3}pH^3$	7.02
40.0	B-2	$k=-0.380451+0.144862pH-0.0174941pH^2+6.89584\times10^{-4}pH^3$	7.24
		$k-D=-0.460493+0.166017pH-0.0188276pH^2+6.77385\times10^{-4}pH^3$	7.23
		$D=0.076144-0.019553pH+1.1144\times10^{-3}pH^2+2.2182\times10^{-5}pH^3$	7.22
40.0	B-3	$k=0.014133-0.013339pH+3.8819\times10^{-3}pH^2-2.7483\times10^{-4}pH^3$	7.16
		$k-D=0.302627-0.142254pH+0.0228920pH^2-1.20695\times10^{-3}pH^3$	7.15
		$D=-0.288494+0.128916pH-0.0190101pH^2+9.32113\times10^{-4}pH^3$	7.14

From the data in Table 3, we establish the non-linear equations of k-pH, D-pH and $(k-D)$-pH for the three strains of petroleum bacterial by non-linear regress analysis. It is obvious that the optimum pH should correspond to the maximum values of the growth rate constants k and $(k-D)$, and the minimum of the death rate constant D. Thus, we obtain the optimum pH from the non-linear equations of k-pH, D-pH and $(k-D)$-pH and the results are listed in Table 4.

From the results, it can be seen that the pH_m is approximately constant at different temperatures when k and $(k-D)$ are the maximum and D the minimum for each bacterium. The values of pH_m are obtained: for B-1, $pH_m=7.01\pm0.02$, for B-2, $pH_m=7.19\pm0.04$, and for B-3, $pH_m=7.10\pm0.03$ at 50.0°C; for B-1, $pH_m=6.97\pm0.05$, for B-2, $pH_m=7.23\pm0.01$, and for B-3, $pH_m=7.15\pm0.01$ at 40.0°C. Therefore, the effect of temperature on the pH of optimum growth is small.

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