ELSEVIER

Thermochimica Acta 338 (1999) 1–6

thermochimica acta

www.elsevier.com/locate/tca

Microcalorimetric investigation of growth conditions of petroleum bacteria

Zhao-Dong Nan[a], Yan Xiang[a], Zhong-Yan Wang[b], Li Li[b], Xian-Cheng Zeng[a,*], Hong-Lin Zhang[c], Hai-Tao Sun[c], Li Yu[c]

[a]*Department of Chemistry, Sichuan University, 610064 Chengdu, China*
[b]*Department of Bioengineering, Sichuan University, 610064 Chengdu, China*
[c]*Department of Chemistry, Qufu Teacher University, 273165 Qufu, China*

Received 3 May 1999; received in revised form 6 July 1999; accepted 8 July 1999

Abstract

The growth power–time curves of three strains of petroleum bacteria (B-1, B-2 and B-3) are determined by using a 2277 thermal activity monitor. A kinetic equation of bacterial growth in limited conditions is used. The equation of describing the power–time curve is derived. The growth rate constants at different temperatures up to 56°C, salinities up to 10.0 (g/100 ml) NaCl and pH ranging from 6.08 to 7.74, are calculated. The lowest growth temperature, optimum growth temperature, highest growth temperature, optimum growth salinity and optimum growth acidity have been obtained, which are 29.1°C, 50.7°C, 54°C, 1.48 and 7.15 respectively for B-1, 28.5°C, 51.4°C, 56°C, 2.38 and 6.91 respectively for B-2, 26.8°C, 50.7°C, 56°C, 3.55 and 7.11 respectively for B-3.

Keywords: Microcalorimetry; Petroleum bacteria; Growth temperature; Optimum growth salinity; Optimum growth acidity

1. Introduction

There are a large number of barrels of crude oil which cannot be recovered by conventional techniques in the world. It is of economic importance to develop recovery methods which can tap this large resource. The use of microorganisms is one option under investigation [1–3]. In microbial enhanced oil recovery (MEOR), the bacteria, salts, and nutrients necessary for microbial growth are pumped into an oil well by water injection. Since few of the environmental parameters of reservoirs can be manipulated, it is necessary to find appropriate bacteria that can grow under reservoirs conditions of temperature, salinity and acidity etc. Thus it is important to investigate the conditions of bacterial growth.

This report describes the effects of temperature, salinity and acidity on growth of three strains of petroleum bacteria isolated from oil well. Calorimetric experiments are carried out to determine the power–time curves of bacterial growth at different temperatures, salinities and acidities. The growth rate constants of bacteria in different conditions are calculated. The relationship of growth rate constant k with respect to temperature T, concentration of NaCl and pH value is established respectively. The lowest growth temperature, optimum growth temperature, highest growth temperature, optimum growth concentration of NaCl and optimum growth acidity of bacteria are obtained.

*Corresponding author.

0040-6031/99/$ – see front matter
PII: S0040-6031(99)00225-7

2. Theory and method

The bacterial growth is often limited by some external constraints, including substrate or product concentrations, pH values and poisoning effects of metabolites. This situation is described by the equation of Verhulst and Pearl ("logistic function") developed from the Malthus equation [4],

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

with $N(t)$ 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,

$$N(t) = \frac{K}{1 + \left[\frac{(K-N_0)}{N_0}\right]\mathrm{e}^{-kt}} \quad (2)$$

with K being the maximum bacterial number during the whole bacterial growth, N_0 the bacterial number at time zero.

When $N(t) = K$, $\mathrm{d}N(t) = 0$, we have

$$\beta = k/K. \quad (3)$$

Let

$$\frac{(K-N_0)}{N_0} = M \quad (4)$$

with M being the final multiple of the initial cell number.

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

$$N(t) = K/[1 + M\mathrm{e}^{-kt}]. \quad (5)$$

Under the assumption that the heat production rate $P(t)$ is proportional to the number of bacteria $N(t)$ [5],

$$P(t) = P_0 N(t) \quad (6)$$

with P_0 being the heat production rate of one cell.

Inserting Eq. (5) into Eq. (6), we have,

$$\ln\left[\frac{P_\mathrm{m}}{P(t)-1}\right] = \ln M - kt \quad (7)$$

with

$$P_\mathrm{m} = P_0 K, \quad (8)$$

P_m being the maximum heat production rate during the whole bacterial growth.

Using the experimental data P_m, $P(t)$ and t obtained from the power–time curves, the growth rate constant k and the final multiple of the initial cell number M can be calculated from linear regression analysis with Eq. (7).

From the growth rate constants at different temperatures T, two equations of k with respect to T can be established at various temperature ranges and the lowest growth temperature and the optimum growth temperature can be obtained.

At the optimum growth temperature, we studied the power–time curves of bacterial growth at different concentrations of NaCl and pH values, and calculated the growth rate constants. From these growth rate constants, equations of k with respect to concentration of NaCl and pH value have been established and the optimum growth concentration of NaCl and the optimum growth pH value have been calculated respectively.

3. Experimental and material

3.1. Instrument

A 2277 thermal activity monitor was used to determine the 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 0.2 μW.

A glass electrode pH-meter was used (mode HM-20S, TOA Electronics Ltd, Japan) with a range of pH = 0.00–14.00.

3.2. Experimental method

In the calorimetric experiment, the stopped-flow operating mode was used. The flow tubing was completely cleaned and sterilized firstly. The procedure was: sterilized distilled water, 0.1 mol L^{-1} HCl, 1 mol L^{-1} NaOH, 75% alcohol solution and sterilized distilled water were pumped by an LKB-2132 microperpex peristalitic pump respectively through the cell, each for 30 min, at a flow rate of 30 ml h^{-1}.

Once the system was cleaned and sterilized, and the baseline had been stabilized, the bacterial sample initially containing 4.98×10^5 cells/ml was pumped into the flow cell system at the same flow rate. When the flow cell (the volume was about 0.6 ml) was full, the pump was stopped. A power–time curve of continuous bacterial growth was recorded. The re-establishment of a stable baseline indicated that the process of bacterial growth finished.

3.3. Material

Three strains of petroleum bacteria, B-1, B-2 and B-3, employed were isolated from the oil well with depths ranging from 800 to 1200 m, temperature about 50°C and pH ranging from 7.0 to 8.0.

Enrichment medium (pH = 7.0–7.2) containing urea (0.3 g), KH_2PO_4 (0.2 g), $MgSO_4$ (0.1 g), yeast extract (0.1 g), petroleum (2.0 g), per 100 ml water was used.

Growth medium: (A) Liquid medium (pH = 7.0) containing NaCl (0.5 g), $NaNO_3$ (0.2 g), $MgSO_4 \cdot 7H_2O$ (0.05 g), $(NH_4)_2$ SO_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 water was used. (B) Liquid medium (pH = 7.0) 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), K_2HPO_4 (1.0 g), yeast extract (0.1 g), glucose (2.0 g), and NaCl in different amounts per 100 ml water was used. (C) Liquid medium containing 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 was used.

The buffer solution consisted of 50 ml 0.1 M KH_2PO_4 and 0.1 M NaOH in different amounts per 100 ml buffer solution.

The media were sterilized at 120°C for 30 min.

4. Results and discussion

4.1. Isolation of petroleum bacteria

Three strains of petroleum bacteria were obtained through enrichment culture. These strains of petroleum bacteria grew on the water–petroleum interface. The inoculum was growth with petroleum as the sole carbon source.

4.2. Determination of the growth temperature

The power–time curves of three strains growth at different temperatures in the medium A have been determined. The power–time curve shows highly reproducible growth patterns under the same conditions. The graphs of three strains growth at 50.0°C are shown in Fig. 1.

The data of P_m, $P(t)$ and t are obtained from Fig. 1. According to Eq. (7), the equations of the power–time curves have been established. For B-1, we have,

$$\ln[235.5/P(t)-1] = 8.116-0.04396t \quad (t< 280 \text{ min}) \tag{9}$$

$r = -0.9983$, $k = 0.04396$ min^{-1}, $M = 3348$ with r being the coefficient of relativity. According to Eqs. (4) and (3), we have $K = 1.67 \times 10^9$ cells/ml, $\beta = 2.63 \times 10^{-11}$ ml/(cells min). For B-2, we have,

$$\ln[99.8/P(t)-1] = 6.219-0.02611t \quad (t<375 \text{ min}) \tag{10}$$

$r = -0.9966$, $k = 0.02611$ min^{-1}. Thus the values of M, K and β can be calculated with the same method as above, $M = 502.2$, $K = 2.51 \times 10^8$ cells/ml, $\beta = 1.04 \times 10^{-10}$ ml/(cells min). For B-3, we have,

$$\ln[240.8/P(t) - 1] = 8.089 - 0.04828\,t \quad (t < 260) \tag{11}$$

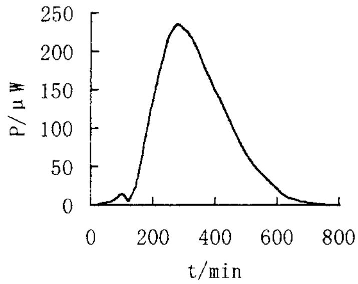
$r = -0.9874$, $k = 0.04828$ min^{-1}, $M = 3257$, $K = 1.62 \times 10^9$ cells/ml, $\beta = 2.98 \times 10^{-10}$ ml/(cells min).

The growth rate constants of bacteria at different temperatures have been calculated by means of the same method. These data are shown in Table 1.

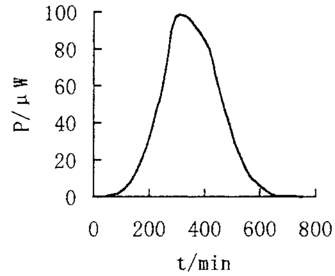
From these results, non-linear equations of k–T have been established during temperatures ranging from 45.0°C to 53.0°C for B-1, 50.0°C to 55.0°C for B-2 and 45°C to 55°C for B-3, respectively. The maximum in the growth rate constant k_{opt} leads to the T_{opt} of the optimum growth temperature. For B-1, we have,

$$k = 1.07747-0.1003577T + 2.75268\times10^{-3}T^2 -2.31786\times10^{-5}T^3 \tag{12}$$

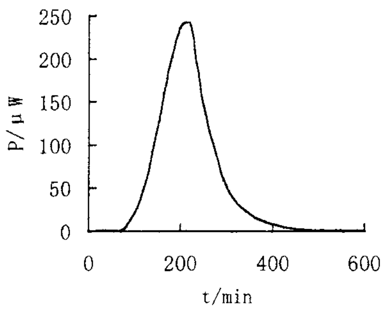
$T_{opt} = 50.7$°C, $k_{opt} = 0.04435$ min^{-1}. For B-2, we have



(B-1)



(B-2)



(B-3)

Fig. 1. Power-time curves of petroleum bacteria growth at 50.0°C.

Table 1
The growth rate constant k ($\times$ 10^2 min^{-1}) of bacteria at different temperatures

Bacteria	T (°C)								
	55.0	53.0	52.0	50.0	45.0	40.0	37.0	35.0	32.0
B-1		4.003	4.302	4.396	2.340	0.9334	0.4865	0.2456	0.07298
		4.003[a]	4.302	4.396	2.340				
B-2	1.800	3.327	4.948	2.611	1.865	1.148	0.8560	0.3865	0.1021
	1.800[b]	3.327	4.948	2.611					
B-3	3.342	4.435	4.769	4.828	2.396	0.9666	0.7541	0.4283	0.1405
	3.339[c]	4.454	4.747	4.834	2.396				

[a] Calculated from Eq. (13).
[b] Calculated from Eq. (14).
[c] Calculated from Eq. (15).

$$k = -359.771783 + 20.4253835T - 0.386155265T^2 + 2.43133501\times10^{-3}T^3 \quad (13)$$

$T_{opt} = 51.4$°C, $k_{opt} = 0.05357$ min^{-1}. For B-3, we have,

$$k = -0.807443 + 9.65771\times10^{-3}T + 6.17087\times10^{-4}T^2 - 9.35855\times10^{-6}T^3 \quad (14)$$

$T_{opt} = 50.7$°C, $k_{opt} = 0.04878$ min^{-1}.

The equations of k with respect to T have been established during temperatures ranging 32–40°C for

B-1, B-2 and B-3 respectively. The value of k equalling to zero leads to the lowest growth temperature T_0. For B-1, we have

$$\sqrt{k} = 8.782\times10^{-3}T - 0.2554 \quad (15)$$

$r = 0.9984$, $T_0 = 29.1°C$ For B-2, we have,

$$\sqrt{k} = 9.739\times10^{-3}T - 0.2771 \quad (16)$$

$r = 0.9814$, $T_0 = 28.5°C$. For B-3, we have,

$$\sqrt{k} = 7.786\times10^{-3}T - 0.2083 \quad (17)$$

$r = 0.9798$, $T_0 = 26.8°C$.

The results of calorimetric experiment indicate that B-1 can grow slowly at 30.0°C, and no growth occurs at 29.0°C during 48 h, B-2 can grow slowly at 29.0°C, no growth occurs at 28.0°C during 48 h, B-3 can grow slowly at 27.0°C, no growth occurs at 26.0°C during 48 h. It demonstrates that the method of calculating the lowest growth temperature described in this paper can be used to calculate the lowest growth temperature.

The experimental results indicate that B-1 can grow slowly at 53.5°C, no growth occurs at 54.0°C during 48 h, B-2 and B-3 can grow slowly at 55.5°C, no growth occurs at 56.0°C during 48 h. The highest growth temperatures are about 54°C, 56°C and 56°C for B-1, B-2 and B-3, respectively. The growth temperature ranges are 29.1–54°C, 28.5–56°C and 26.8–56°C for B-1, B-2 and B-3, respectively.

4.3. Determination of the optimum growth salinity

The power-time curves of three strains growth at the optimum temperatures and different salinities in the medium B have been determined respectively. The power–time curve shows highly reproducible growth patterns under the same conditions.

The growth rate constants of three strains have been calculated with the same method. These data are shown in Table 2.

The equations of k with respect to the concentration of NaCl (g/100 ml) m have been established for B-1, B-2 and B-3 respectively. The maximum growth rate constant k_{opt} leads to the optimum growth concentration of NaCl m_{opt} For B-1, we have

$$k = 0.035811 + 0.021164\text{m} - 9.651\times10^{-3}\text{m}^2 + 1.130\times10^{-3}\text{m}^3 \quad (18)$$

$m_{opt} = 1.48$, $k_{opt} = 0.04966\ \text{min}^{-1}$. For B-2, we have

$$k = 0.051057 - 7.80\times10^{-4}\text{m} + 1.7153\times10^{-3}\text{m}^2 - 3.107\times10^{-4}\text{m}^3 \quad (19)$$

$m_{opt} = 3.44$, $k_{opt} = 0.05602\ \text{min}^{-1}$. For B-3, we have

$$k = 0.047413 + 1.6312\times10^{-3}\text{m} + 5.5489\times10^{-4}\text{m}^2 - 1.4715\times10^{-4}\text{m}^3 \quad (20)$$

$m_{opt} = 3.55$, $k_{opt} = 0.05361\ \text{min}^{-1}$.

No growth occurs with m equalling to 10.0 for three strains.

From Table 2 the results indicate that the effect on three strains growth is small during concentrations of NaCl from 0.5 to 2.0 for B-1, 0.5 to 4.0 for B-2, 0.5 to 4.0 for B-3, respectively.

4.4. Determination of the optimum growth acidity

The power-time curves of three strains growth in the medium C have been determined at the optimum

Table 2
Growth rate constants of bacteria $k(\times 10^2\ \text{min}^{-1})$ with different NaCl concentrations

Bacteria	NaCl (g/100 ml)							
	0.1	0.5	1.0	1.5	2.0	4.0	6.0	8.0
B-1	2.139	4.412	4.846	4.965	4.858	3.839	3.593	2.833
		4.412[a]	4.845	4.966	4.858	3.839		
B-2	3.271	5.102	5.186	5.241	5.403	5.548	4.101	2.402
		5.106[b]	5.168	5.270	5.387	5.549	4.101	
B-3	4.103	4.852	4.904	5.083	5.177	5.336	4.540	3.615
		4.835[c]	4.945	5.061	5.172	5.340	4.539	

[a] Calculated from Eq. (18).
[b] Calculated from Eq. (19).
[c] Calculated from Eq. (20).

Table 3
The growth rate constants $k(\times 10^2 \text{ min}^{-1})$ of two strains at different acidities

Bacteria	pH						
	6.06	6.48	6.72	6.97	7.20	7.40	7.74
B-1	3.357	3.421	3.446	4.567	4.744	4.448	3.305
			3.447[a]	4.561	4.753	4.442	3.306
B-2	3.304	3.386	3.466	4.500	3.276	2.687	
			3.466[b]	4.500	3.276	2.687	
B-3	3.617	3.728	3.847	4.054	4.123	2.509	
			3.847[c]	4.054	4.123	2.509	

[a] Calculated from Eq. (21).
[b] Calculated from Eq. (22).
[c] Calculated from Eq. (23).

temperatures and different acidities respectively. The power–time curve shows highly reproducible growth patterns under the same conditions.

The growth rate constants of three strains have been calculated with the same method . The data are shown in Table 3.

The equations of k with respect to pH have been established for B-1, B-2 and B-3 respectively. The maximum growth rate constant k_{opt} leads to the optimum growth acidity pH_{opt}. For B-1, we have,

$$k = -13.633561 + 5.3181192\,\text{pH} - 0.6847239\,\text{pH}^2 + 0.02916753\,\text{pH}^3 \qquad (21)$$

$\text{pH}_{\text{opt}} = 7.15$, $k_{\text{opt}} = 0.04768\ \text{min}^{-1}$. For B-2, we have,

$$k = -134.600971 + 56.6906404\,\text{pH} - 7.9481001\,\text{pH}^2 + 0.37104185\,\text{pH}^3 \qquad (22)$$

$\text{pH}_{\text{opt}} = 6.91$, $k_{\text{opt}} = 0.04602\ \text{min}^{-1}$. For B-3, we have,

$$k = 90.54700 - 39.111870\text{pH} + 5.630882\text{pH}^2 - 0.27007572\text{pH}^3 \qquad (23)$$

$\text{pH}_{\text{opt}} = 7.11$, $k_{\text{opt}} = 0.04243$.

From Table 3 the results indicate that the effect on three strains growth is small during pH value from 6.06 to 6.72.

The growth temperatures, optimum growth salinity and optimum growth acidity have been obtained for three strains of petroleum bacteria. These obtained data are very useful theoretically and practically for MEOR.

References

[1] C.E. Zebell, Recovery of hydrocarbons, 1953, US Patent 2641556.
[2] I. Lazar, P. Constantinescu, Field trials results of microbial enhanced oil recovery, in: J.E. Zajic, E.C. Donaldson (Eds.), Microbes and Oil Recovery, 1985, Bioresource Publication, Elpaso, TX, pp. 122–143.
[3] M.J. Brown, V. Moses, J.P. Robinson, D.G. Springham, Microbial enhanced oil recovery: progress and prospets, Grit. Rev. Biotechnol. 3 (1986) 159–197.
[4] R. Holzel, C. Motzkus, I. Lamprecht, Thermochim. Acta 239 (1994) 17.
[5] X. Changli, T. Houkuan, S. Zhaohua, Q. Songsheng, Thermochim. Acta 123 (1988) 33.