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Thermokinetic investigation of effects of carbon source on petroleum bacterial growth

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Abstract

The growth power–time curves of a strain of petroleum bacteria, B-2, in various kinds of cultures containing different kinds of carbon sources, glucose, *n*-tetradecane, *n*-hexadecane and *n*-octadecane, and different kinds of microemulsions have been determined by using a 2277 Thermal Activity Monitor. The curves showed a single peak for cultures containing a single carbon source, glucose, and two peaks for cultures containing two kinds of carbon sources, glucose and one of the *n*-alkanes. The first peak indicated that bacteria grew by consuming glucose and the second peak indicated that bacteria grew by consuming *n*-alkane. The curves were complex when the bacterium grows in a microemulsion culture. According to a kinetic equation of bacterial growth under limited conditions, the rate constants of bacterial growth were obtained. The results showed that the microemulsion culture was more appropriate to bacteria to grow on *n*-alkanes. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Thermokinetics; Power–time curve; Petroleum bacteria; Carbon source; Microemulsion

1. Introduction

It is well known that microbial enhanced oil recovery (MEOR) is of more economic importance compared to the conventional techniques. Thus, the use of microorganism to enhance oil recovery has been extensively investigated [1–4]. Bacteria are not soluble in water, they exist in water as a suspension. Here, they may concentrate at the interface between water and hydrocarbon. Therefore, the degradation of hydrocarbons contained in crude oil takes place at the interface between water and hydrocarbon [5]. Setti et al. studied the effect of the molecular weight of the *n*-alkane on its degradation [6]. However, little has been reported on MEOR by using bacterial growth in microemulsion culture.

We recently investigated the growth conditions of petroleum bacteria isolated from the oil well with glucose used as the only carbon source [7,8]. Moving along these lines, the effects of carbon sources and microemulsions on petroleum bacterial growth have been studied at the optimum growth conditions. This paper presents a detailed account concerning the effects of glucose, *n*-alkanes and microemulsions on growth of a strain of petroleum bacteria B-2.

2. Theory and method

According to the equation of Verhulst and Pearl (“logistic function”) [9], we have,

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

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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.

Under the assumption that the heat production rate $P(t)$ is proportional to the number of bacteria $N(t)$ [10],

$$P(t) = P_0 N(t) \tag{2}$$

with P_0 being the heat production rate of one cell.

We obtain [7],

$$\ln\left[\frac{P_m}{P(t) - 1}\right] = \ln M - kt \tag{3}$$

with

$$P_m = P_0 K \tag{4}$$

$$M = \frac{K - N_0}{N_0} \tag{5}$$

P_m and K being the maximum heat production rate and maximum bacterial number during the whole bacterial growth, respectively, M the final multiple of the initial cell number, N_0 the bacterial number at time zero.

Using the experimental data of P_m, $P(t)$ and t obtained from the power–time curves, the growth-rate constant k can be calculated from linear regression analysis with Eq. (3).

3. Experimental and material

3.1. Instrument

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 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. The performance of this instrument and the details of its construction have been described previously [11].

3.2. Experimental method

In the calorimetric experiment, the ampoule mode was used with two ampoules of 4 ml capacity each. One was filled with 1 ml of the bacterial sample initially containing 4.98×10^5 cells/ml and the other with only 1 ml of medium.

Once thermal balance of the system was established, a recorder began to record a power–time curve of continuous bacterial growth. The re-establishment of a stable baseline indicated that the process of bacterial growth had finished. The experimental temperature was 51.0 °C.

3.3. Phase diagram

NaCl, *n*-pentanol, *n*-octanol and *n*-octadecane were analytical-grade commercial products. Nonionic surfactant, Tween 80, Jining Chemical Engineering Institute, was used. According to the method of [12], the phase diagram has been determined. The experimental temperature was 51 ± 0.1 °C.

3.4. Material

The strain of petroleum bacteria, B-2, used was isolated from an oil well as in references [7,8].

Growth medium: (1) Liquid medium(pH = 7.0) containing NaCl (3.4 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); and one of the carbon sources, (a) glucose (2.0 g), (b) glucose (2.0 g) and *n*-tetradecane (2.0 g), (c) glucose (2.0 g) and *n*-hexadecane (2.0 g), (d) glucose (2.0 g) and *n*-octadecane (2.0 g), per 100 ml water was used. (2) Liquid medium of microemulsion (pH = 7.0) containing NaCl (3.4 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), the ratio of Tween 80, *n*-alcohol and *n*-octadecane showed in Table 1 and as points 1–6 in Fig. 1, per 100 ml water

Table 1
Mass ratio (given as percentage) of H_2O, Tween 80, *n*-pentanol, *n*-octanol and *n*-octadecane

	Point					
	1	2	3	4	5	6
H_2O	80.0	50.0	10.0	80.0	50.0	20.0
Tween 80	14.4	36.0	32.4	12.8	32.0	51.2
n-Pentanol	1.50	3.75	39.6	–	–	–
n-Octanol	–	–	–	3.20	8.00	12.8
n-Octadecane	4.10	10.25	18.0	4.00	10.0	16.0

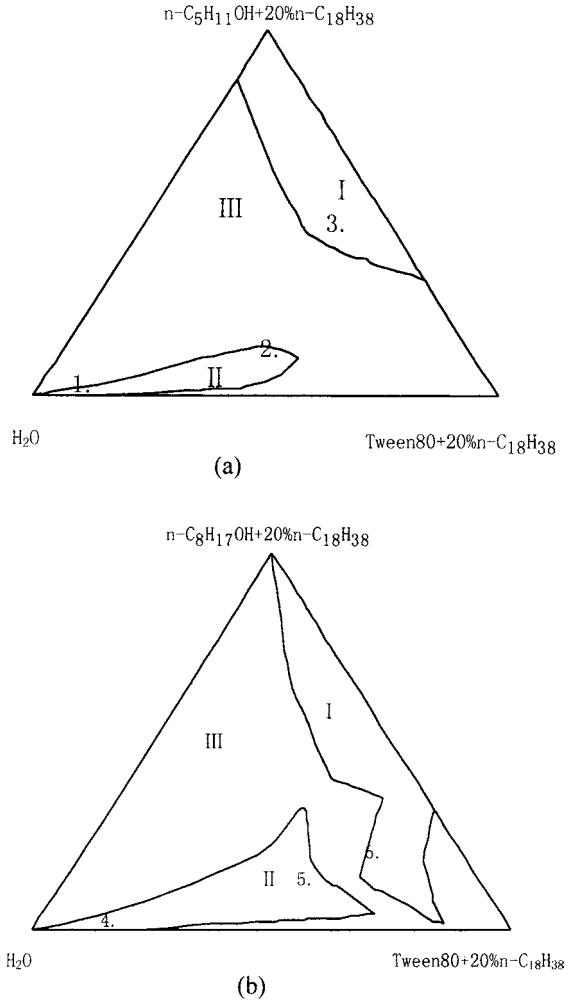

Fig. 1. Phase diagrams of the systems: (a) Tween 80 + 20% *n*-octadecane/*n*-pentanol + 20% *n*-octadecane/0.60 mol/water; (b) Tween 80 + 20% *n*-octadecane/*n*-octanol + 20% *n*-octadecane/water. (I) W/O microemulsion; (II) O/W microemulsion; (III) two-phase region.

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

4. Results and discussion

4.1. Determination of the phase diagram

The structure of microemulsions has been investigated extensively ever since their introduction by Hoar and Schulman [13]. Microemulsions generally contain hydrocarbons in ternary systems, water–alcohol–surfactant [14–16]. The systems investigated in this paper contain *n*-octadecane, *n*-pentanol or *n*-octanol, a nonionic surfactant, Tween 80, and NaCl aqueous solution. The nonionic surfactant, Tween 80, has been used to enhance oil recovery [17]. The optimum NaCl concentration of B-2 growth is about 0.60 mol/l [7]. According to our investigation, the effect of NaCl concentration on the microemulsion is small because of the nonionic surfactant used in this paper. Then, the growth culture of B-2 is in microemulsions as the liquid medium 2.

The phase diagrams of the systems, Tween 80/*n*-pentanol/*n*-octadecane/water and Tween 80/*n*-octanol/*n*-octadecane/water, have been determined, respectively. The phase diagrams are shown in Fig. 1.

4.2. Determination of the growth power–time curve

The growth power–time curves of B-2 have been determined in media 1 and 2, respectively. The power–time curves show highly reproducible growth patterns under the same conditions. The graphs are shown in Figs. 2 and 3. B-2 does not grow in the microemulsion, the composition of which is as at point 3.

4.3. Establishment of equations of growth power–time curves

The data of P_m, $P(t)$ and t are obtained from Figs. 2 and 3. According to Eq. (3), the equations of the power–time curves have been established. The equations are shown in Tables 2 and 3.

4.4. Discussion

Fig. 1 shows that the systems, Tween 80/*n*-pentanol/*n*-octadecane/water and Tween 80/*n*-octanol/*n*-octadecane/water, contain W/O (water in oil) microemulsion, O/W (oil in water) microemulsion and two-phase region. Points 1–6 selected are contained in the area of W/O microemulsion and O/W microemulsion, respectively, so as to study the effect of different kinds of microemulsions on petroleum bacterial growth.

Fig. 2 shows that the growth power–time curves exhibit a single peak at media containing only a single carbon source, glucose, and double peaks at media

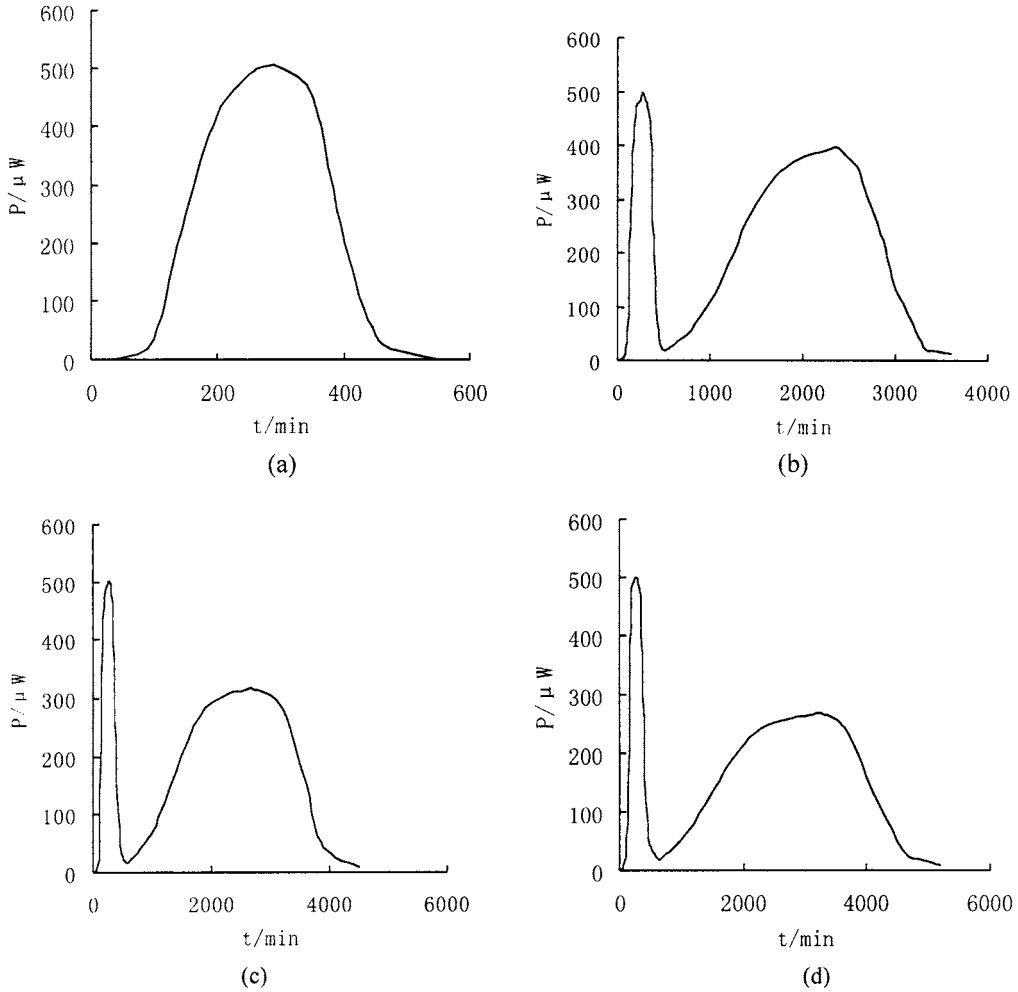

Fig. 2. Growth power–time curves of B-2 in medium 1 at different carbon source: (a) glucose, (b) glucose and *n*-tetradecane; (c) glucose and *n*-hexadecane; (d) glucose and *n*-octadecane.

containing two kinds of carbon sources, glucose and one of the *n*-alkanes. According to reference [18], bacteria always make use of the most suitable carbon source firstly. Glucose is more suitable for bacterial growth than *n*-alkane. Therefore, glucose is used as carbon source firstly. When the glucose is completely consumed in the medium, the *n*-alkane begins to be used. The equations of growth power–time curves in Table 2 demonstrate that the first peak corresponds to bacteria growth on glucose, while the second peak corresponds to bacteria growth on *n*-alkane. The reason, why the growth power–time curves of bacteria do not return to the baseline when B-2 grows in the culture containing glucose and one of the *n*-alkanes as carbon sources, may be that these media include two phases, one of which is an aqueous solution and the other *n*-alkane, which can be used slowly as carbon source of bacterial growth at the interface of aqueous solution and *n*-alkane. Thus, *n*-alkane can be used over a long period of time and it needs a long period of time for the growth power–time curves of bacteria to return to the baseline. From the data in Table 2, the bacterial growth-rate constants are greater during bacteria making use of glucose than that of bacteria consuming *n*-alkane, which indicates that glucose is more suitable as carbon source than *n*-alkane. The lower the mole-

cular weight, the greater the growth-rate constant with regard to one of the *n*-alkanes as carbon source. According to reference [6], with regard to *n*-alkanes, lower the molecular weight, more degradable the compound is. It indicates that the results obtained in this paper are reasonable. Glucose is always the carbon source that is most easily degraded. Thus, only after the exhaustion of glucose other sources are catabolized.

From Fig. 3, it can be seen that the curves do not exhibit peaks corresponding to the different carbon sources. The reason may be that the glucose and *n*-octadecane locate the same phase. The curves keeping high thermal power for a long period of time shows that the *n*-octadecane has been made full use of as the carbon source of bacterial growth. That means that the microemulsion makes better effect on MEOR than the culture containing two phases. From Fig. 3 and

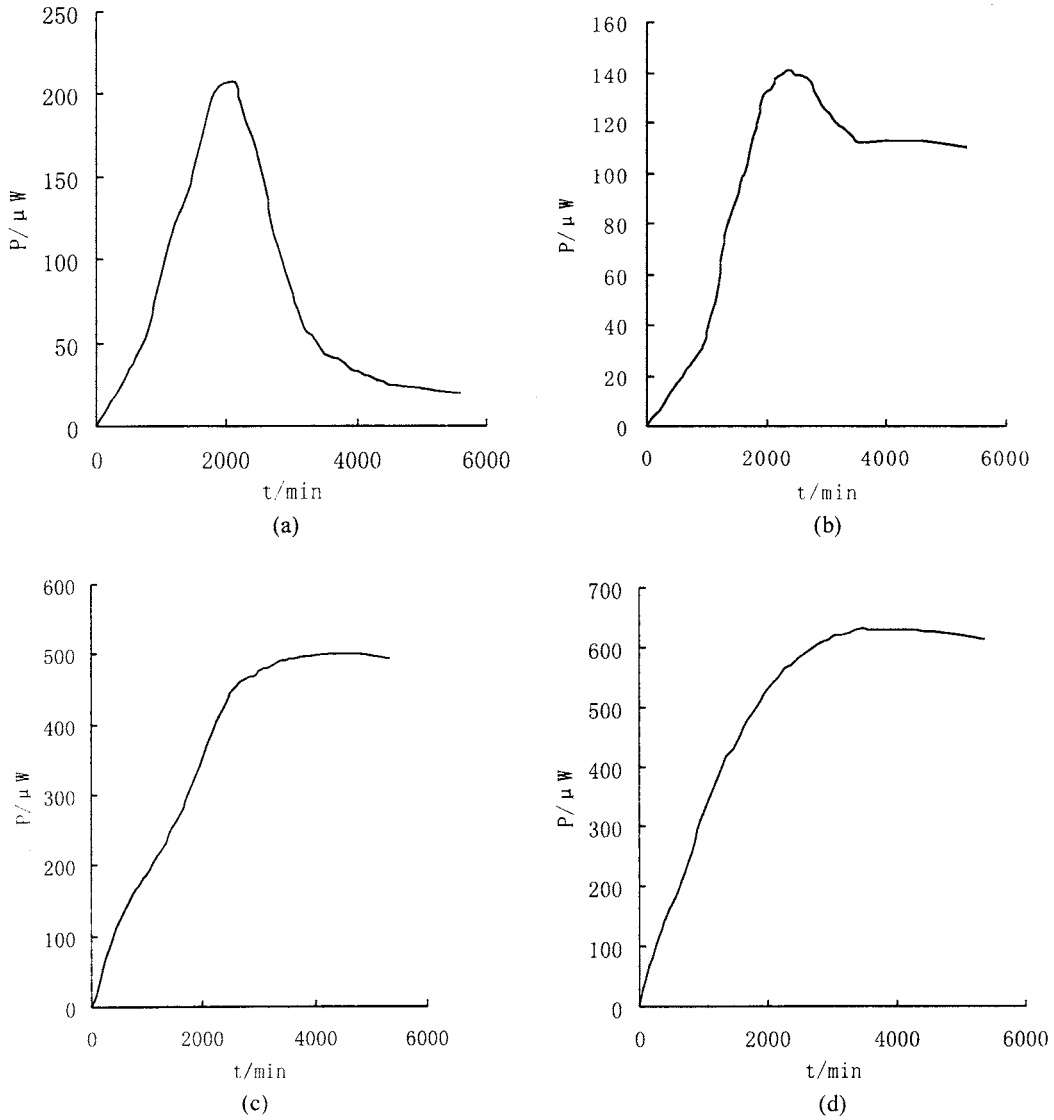

Fig. 3. Power–time curves of petroleum bacterial strain B-2 growth in medium 2, composition of the medium as points (a) 1, (b) 2, (c) 4, (d) 5 and (e) 6.

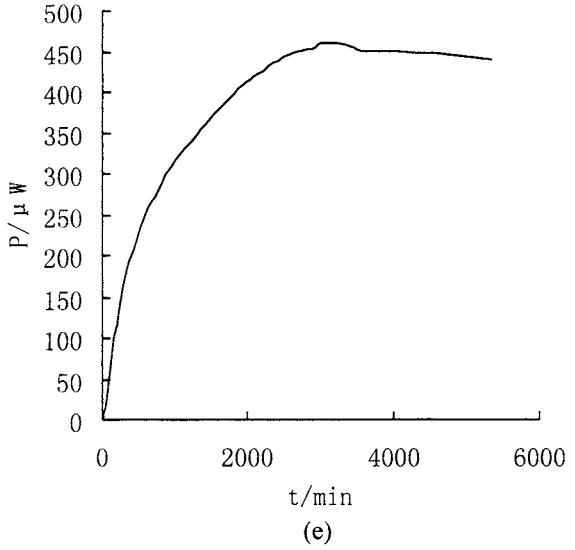

Fig. 3. (*Continued*).

Table 3, it can be seen that the shapes and the equations of the growth curves of B-2 show that B-2 growth is inhibited by the microemulsions cultures by consuming glucose as carbon the source, on the contrary for the culture 1. The reasons may be that the glucose and *n*-octadecane locate the same phase, then, the *n*-octadecane inhibits the glucose being used as the carbon source. The thermal power and rate constant of bacterial growth become smaller when the content of *n*-pentanol increases in the culture, the composition of which is similar to that at points 1–3. B-2 cannot grow at point 3. The thermal power and rate constant of bacterial growth become greater when the content of *n*-octanol increases in the O/W region, the composition of which is as at points 4 and 5, and become smaller when the content of *n*-octanol increases in the W/O region, the composition of which is as at point 6. Then, the carbon number contained in the alcohol and the content of the alcohol affect bacterial growth. The smaller the carbon number contained in the alcohol, more the bacterial growth is inhibited as we know that alcohol is always used to kill bacteria. The effect of the content of the alcohol in the culture on bacterial growth is different for different kinds of alcohol.

From the above results, the conclusion can be obtained that the microemulsion culture is more suitable for petroleum bacterial growth by making use of paraffin, then, the microemulsion culture is better for MEOR, and O/W microemulsion is most suitable for petroleum bacterial strain B-2.

Table 2
Equations of power–time curves of B-2 growth in liquid medium 1

Carbon source	Equation of power–time curve	Growth-rate constant (min^{-1})	Correlation (min^{-1})
Glucose	$\ln[(502.5/P(t)) - 1] = 8.25 - 0.0564t$, $t < 275$ min	0.0564	−0.990
Glucose and tetradecane	$\ln[(500.2/P(t)) - 1] = 8.22 - 0.0561t$, $t < 275$ min	0.0561	−0.987
	$\ln[(396.8/P(t)) - 1] = 5.02 - 0.00403t$, 550 min $\leq t < 2375$ min	0.00403	−0.983
Glucose and hexadecane	$\ln[(501.8/P(t)) - 1] = 8.23 - 0.0562t$, $t < 275$ min	0.0562	−0.989
	$\ln[(317.5/P(t)) - 1] = 4.84 - 0.00362t$, 620 min $\leq t < 2650$ min	0.00362	−0.980
Glucose and octadecane	$\ln[(499.8/P(t)) - 1] = 8.19 - 0.0559t$, $t < 275$ min	0.0559	−0.988
	$\ln[(267.2/P(t)) - 1] = 4.21 - 0.00281t$, 715 min $\leq t < 3255$ min	0.00281	−0.976

Table 3
Equations of power–time curves of B-2 growth in liquid medium 2

Composition of the medium	Equation of power–time curve	Growth-rateconstant	Correlation
Point 1	$\ln[(208.0/P(t)) - 1] = 3.57 - 0.00387t$, $t < 2125$ min	0.00387	−0.972
Point 2	$\ln[(141.0/P(t)) - 1] = 3.08 - 0.00216t$, $t < 236$ min	0.00216	−0.988
Point 4	$\ln[(500.0/P(t)) - 1] = 5.41 - 0.00580t$, $t < 4550$ min	0.00580	−0.991
Point 5	$\ln[(632.0/P(t)) - 1] = 6.40 - 0.00699t$, $t < 3460$ min	0.00699	−0.979
point 6	$\ln[(461.0/P(t)) -] = 4.92 - 0.00501t$, $t < 3020$ min	0.00501	−0.989

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