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Determination of thermograms of bacterial growth and study of optimum growth temperature

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Abstract

In this paper, we have determined bacterial growth thermograms by using the 2277 thermal activity monitor. We have studied the multiplication curve of bacteria at different temperatures and calculated the rate constant and optimum growth temperature.

INTRODUCTION

In any living system, the various metabolic events occurring within the cells are all reactions producing heat. We can study the metabolic process of living cells by continuous measurement of the heat effect of the growing cells with a calorimeter. One can see that the time dependence of the observed thermal effect (the thermogram curve) reflects time dependent changes in growth patterns. It has recently been demonstrated that calorimetric methods can be used for fundamental growth studies of bacteria [1]. For example, Boling et al. have examined growth patterns of *Enterobacter aerogenes et* [2].

In this paper, we have used a 2277 thermal activity monitor to determine thermograms of the growth process of *Vibrio alginolyticus* and *Vibrio bemolyticus*; the thermograms reflect changes in the bacterial growth processes. From these growth curves we are able to calculate the multiplication rate constant and optimum growth temperature.

INSTRUMENT

A new type of heat-flow microcalorimeter, the 2277 thermal activity monitor was used in this experiment. The heat produced in a thermally uninsulated vessel flowed away in an effort to establish thermal equilibrium

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with its surroundings. Exceptional thermal stability was achieved by utilising a 25 l water thermostat surrounding the reaction measuring vessels and acting as an infinite heat sink. Reactions could be studied in the temperature range 5–80°C (the working temperature range of the thermostat). Four individual measuring vessels could be housed in the water thermostat, where they were maintained at the given temperature, constant to within $\pm 2 \times 10^{-4}$°C, allowing fractions of a microwatt to be measured routinely.

This system was very sensitive: the detection limit was 0.15 μW and the baseline stability (over a period of 24 h) was 0.2 μW.

The thermal activity monitor had three operating modes: ampoule mode; flow-through mode; flow-mix mode.

In this experiment the flow through mode was used. The sample was pumped through the flow cell by a microperpex pump.

EXPERIMENTAL METHOD

The experimental set-up is shown in Fig. 1.

The complete cleaning and sterilization procedure for the flow tubing was as follows.

(a) Sterilised distilled water was pumped through the system for 30 min at a flow rate of 30 ml h^{-1}.

(b) 0.1 M HCl was pumped through the system for 30 min at a flow rate of 30 ml h^{-1}.

(c) Alcohol solution (75 vol.%) was pumped through the system for 30 min at a flow rate of 30 ml h^{-1}.

(d) Sterilised distilled water was again pumped through the system for 30 min, at a flow rate of 30 ml h^{-1}.

Once the system had been cleaned and sterilised, sterilised distilled water was pumped through the system at a flow rate of 10 ml h^{-1} and the baseline was determined. When a stable baseline had been obtained, the bacterial sample was pumped into the flow cell system and the monitor used to record the thermogram of continuous bacterial growth. When the recording pen returned to the baseline and became stabilised the process of bacterial growth was complete.

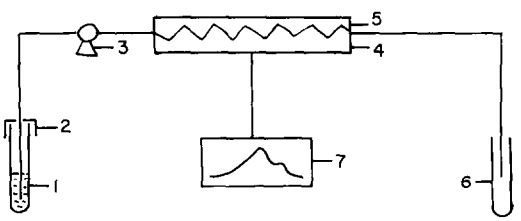

Fig. 1. Schematic diagram of operation for slopped flow measurement: 1, bacterial solution; 2, cover; 3, pump; 4, calorimeter; 5, reaction vessel; 6, waste solution; 7, recorder.

MATERIALS

The bacteria employed were *Vibrio alginolyticus* and *Vibrio bemolyticus*.

A soluble medium (pH = 7.2–7.4) was used, containing in every 200 ml NaCl (1 g), peptone (2 g) and beef extract (1 g).

CALCULATION OF THE MULTIPLICATION RATE CONSTANT

In the logarithmic phase of growth, bacterial numbers and culture time are in accordance with an exponential law [3] so if the bacterial number is n_0 at time t_0 and n_t at time t, then

$$n_t = n_0 \exp[k_a(t - t_0)] \quad (1)$$

If the power given out by every bacterial is y, then $P_0 = n_0 y$ and $P_t = n_t y$. Therefore

$$P_t = P_0 \exp[k_a(t - t_0)] \quad (2)$$

When $t_0 = 0$ (at the starting point of the logarithmic phase of growth)

$$P_t = P_0 \exp(k_a t) \quad (3)$$

or

$$\ln P_t = \ln P_0 + k_a t \quad (4)$$

where k_a is the multiplication rate constant.

Data for P_t, t and the rate constant k_a at 28°C are shown in Table 1.

TABLE 1

P_t and t values at 28°C

Vibrio alginolyticus			*Vibrio bemolyticus*		
t (min)	P_t	$\ln P_t$ [a]	t (min)	P_t	$\ln P_t$ [b]
25	1.3	0.2639	25	1.2	0.1823
50	3.0	1.0986	50	2.0	0.693
60	4.2	1.4364	75	3.2	1.1632
75	7.0	1.9411	100	6.6	1.8871
90	11.5	2.4414	125	12.0	2.4849
100	16.0	2.7726	140	17.2	2.8449
120	31.0	3.4340	150	21.0	3.0445

[a] $\ln P_t = -0.5689 + 0.0334t$ ($r = 0.9999$). [b] $\ln P_t = -0.4732 + 0.0235t$ ($r = 0.9985$).

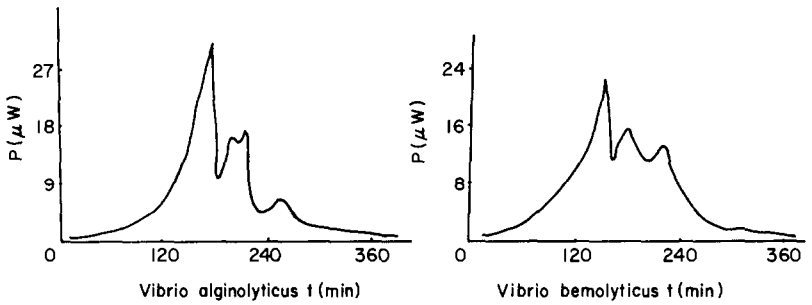

Fig. 2. Thermograms of bacteria at 28°C.

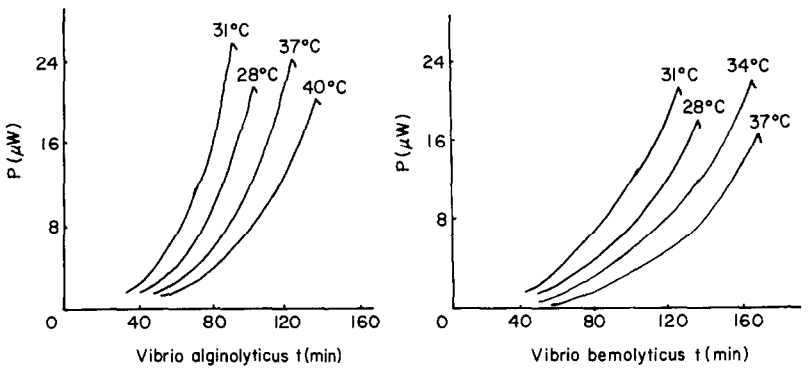

Fig. 3. Thermogram curves of bacteria in exponential phase of growth at various temperatures.

Thermograms of *Vibrio alginolyticus* and *Vibrio bemolyticus* are given in Figs. 2 and 3.

CALCULATION OF OPTIMUM GROWTH TEMPERATURES

We have determined the multiplication rate constants k_a of *Vibrio alginolyticus* and *Vibrio bemolyticus* at various temperatures (Table 2).

TABLE 2

Growth rate constant of bacteria

Bacterial name	Rate constant k_a (min^{-1})				
	28°C	31°C	34°C	37°C	40°C
Vibrio alginolyticus	0.0334 (0.9999) [a]	0.0372 (0.9990) [a]		0.0363 (0.9998) [a]	0.0334 (0.9901) [a]
Vibrio bemolyticus	0.0235 (0.9985) [a]	0.0282 (0.9950) [a]	0.0297 (0.9733) [a]	0.0258 (0.9954) [a]	

[a] Correlation coefficient.

From these results, a non-linear equation of the form $k_a = aT^2 + bT + C$ can be established.

The corresponding nonlinear equation for *Vibrio alginolyticus* is

$$k_a = -1.1726 \times 10^{-4} T_a^2 + 0.071983 T_a - 11.00924$$

when $\partial k_a / \partial T_a < 0$, $\partial^2 k_a / \partial T_a^2 = 0$ and $T_a = 306.94$ K.

For *Vibrio bemolyticus*

$$k_b = -2.4606 \times 10^{-4} T_b^2 + 0.15068 T_b - 23.03996$$

when $\partial k_a / \partial T_b < 0$, $\partial^2 k_a / \partial T_b^2 = 0$ and $T_b = 306.19$ K.

T_a and T_b are optimum growth temperatures.

CONCLUSION

These thermograms contain much information concerning the kinetics of metabolic processes. From them we can measure the multiplication rate constant k_a of bacterial growth. From the rate constant at various temperatures we have determined the optimum growth temperature.

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