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Determination of the thermograms and establishment of the experimenal law for bacterial growth and study of kinetic properties

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Abstract

In this paper, we have recorded thermograms of bacterial growth using a 2277 thermal activity monitor. We have established the experimental law of bacterial growth and calculated the rate constant of the multiplication curve.

INTRODUCTION

The various metabolic events occurring within cells are all heat-producing reactions. We can study the metabolic process of living cells by continuous measurement of the heat effect of the growing cells using a calorimeter, and by observing the thermogravimetric curve of bacterial growth. In general, the metabolism of bacterial cells is very complicated: simple metabolic processes are studied here.

A heat-flow microcalorimeter was used to record the thermograms for the growth processes of *Bacillus subtilis.*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* under limited conditions. We have established the experimental law of bacterial growth and these growth curves can be used to calculate the multiplication rate constant.

INSTRUMENTAL

A new type of heat-flow microcalorimeter, the 2277 thermal activity monitor, was used in this experiment. The instrument can be used within the range 10–80°C, the working range of the thermostat. It was maintained at a given temperature, constant to within $\pm 2 \times 10^{-4}$ °C.

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This system is very sensitive: the detection limit is 0.15 μW and the baseline stability (over a period of 24 h) is 0.2 μW. There are three operating modes, ampoule mode, flow-through mode and 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

First, the flow tubing was cleaned and sterilized. Sterilized distilled water was pumped through the system for 30 min at a flow rate of 30 ml h^{-1}; then 0.1 M HCl was pumped through for 30 min at a flow rate of 30 ml h^{-1}; then alcohol solution (75%) for 30 min at a flow rate of 30 ml h^{-1}.

Once the system was cleaned and sterilized, sterilized distilled water was pumped through the system at a flow rate of 10 ml h^{-1} for 30 min and the baseline was determined. After a stable baseline had been obtained, the bacterial sample was pumped into the flow cell system and a thermogram of continuous bacterial growth was recorded. The re-establishment of a stable baseline indicated that the process of bacterial growth was complete.

MATERIALS

The following bacteria were employed: *B. subtilis, S. epidermidis* and *P. aeruginosa.*

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

ESTABLISHMENT OF THE EXPERIMENTAL LAW AND CALCULATION OF THE RATE CONSTANT

For non-limited conditions, the model of bacterial growth follows the exponential law [1]

$$dN(t)/dt = \mu N(t) \tag{1}$$

For limited conditions, the model of bacterial growth follows an experimental equation [2]. In the growth phase, bacterial numbers and time are related according to

$$dN(t)/dt = \mu N(t) - \beta N^2(t) \tag{2}$$

where μ is the growth rate constant, β is the deceleration rate constant, and

$N(t)$ represents bacterial numbers at time t. If the power given out by every bacterium is P_0, then

$$P(t) = P_0 N(t) \tag{3}$$

So we have

$$\frac{\mathrm{d}\left(\frac{P(t)}{P_0}\right)}{\mathrm{d}t} = \mu\left(\frac{P(t)}{P_0}\right) - \beta\left(\frac{P(t)}{P_0}\right)^2$$

or

$$\frac{\mathrm{d}P(t)}{\mathrm{d}t} = \mu P(t) - \left(\frac{\beta}{P_0}\right)P(t)^2 \tag{4}$$

The integral equation is given by

$$P(t) = \frac{1}{[1/P_0 - \beta/\mu P_0]\mathrm{e}^{-\mu t} + \beta/\mu P_0} \tag{5}$$

or

$$P(t) = \frac{1}{a\mathrm{e}^{-\mu t} + b} \tag{6}$$

where $a = 1/P_0 - \beta/\mu P_0$ and $b = \beta/\mu P_0$.

Using the data for $P(t)$ and t obtained from the bacterial growth curve, a non-linear equation can be fitted and the growth rate constant μ and deceleration rate constant β obtained. The data for $P(t)$, $\hat{P}(t)$ and t at 37°C are shown in Table 1. The thermograms are displayed in Fig. 1.

TABLE 1

$P(t)$, $\hat{P}(t)$ and t values at 37°C [a]

B. subtilis			*S. epidermidis*			*P. aeruginosa*		
t/min	$P(t)/\mu$W	$\hat{P}(t)/\mu$W	t/min	$P(t)/\mu$W	$\hat{P}(t)/\mu$W	t/min	$P(t)/\mu$W	$\hat{P}(t)/\mu$W
50	0.6	0.65	20	0.15	0.13	50	0.3	0.74
75	1.4	1.52	40	0.28	0.24	75	1.5	1.47
100	3.2	3.43	60	0.48	0.44	100	2.7	2.85
110	4.5	4.67	80	0.80	0.78	125	5.1	5.39
125	7.0	7.24	100	1.60	1.41	140	7.4	7.74
140	10.6	10.75	120	2.70	2.52	150	9.4	9.73
150	13.8	13.58	140	4.70	4.46	160	11.9	12.08
160	16.6	16.67	160	7.8	7.72	170	15.0	14.80
165	18.8	18.27	180	13.2	12.89	175	16.5	16.29
167.5	19.0	19.08	200	20.2	20.43	177.5	17.0	17.06

[a] $P(t)$ are experimental data; $\hat{P}(t)$ are data calculated from the model.

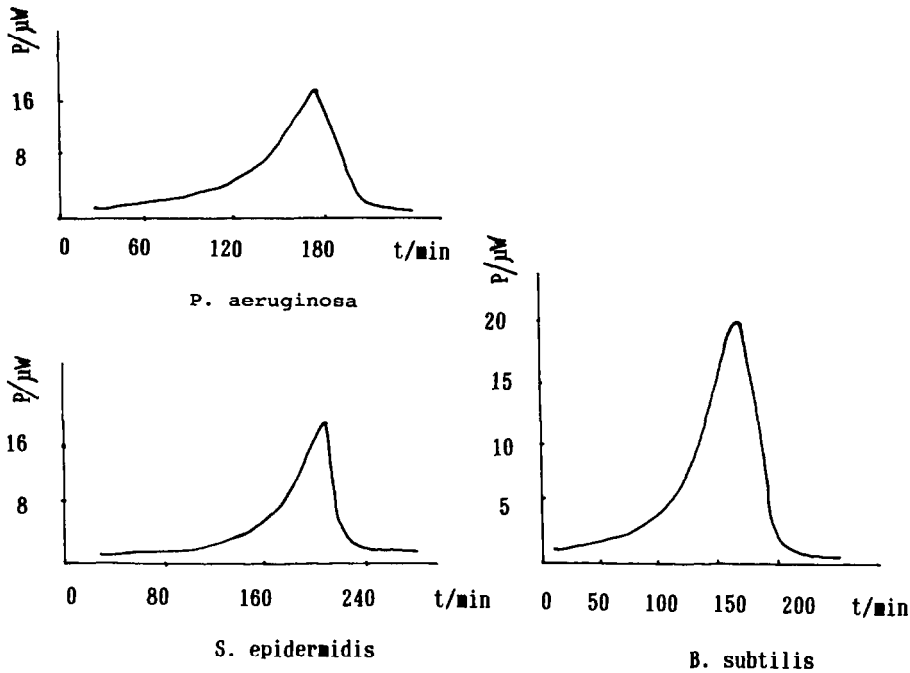

Fig. 1. Thermograms for bacterial growth at 37°C.

The corresponding non-linear equations are: for *B. subtilis*

$$P(t) = \frac{1}{8.5e^{-0.0347t} + 0.027} \qquad t \leqslant 167.5 \text{ min}$$

$\mu = 0.0347, \qquad \beta = 0.00799.$

For *S. epidermidis*

$$P(t) = \frac{1}{13.8e^{-0.0299t} + 0.014} \qquad t \leqslant 200 \text{ min}$$

$\mu = 0.0299, \qquad \beta = 0.00578.$

For *P. aeruginosa*

$$P(t) = \frac{1}{5.3e^{-0.0277t} + 0.02} \qquad t \leqslant 177.5 \text{ min}$$

$\mu = 0.0277, \qquad \beta = 0.00295.$

CONCLUSIONS

These thermograms contain much information. From them, we have established a new experimental law for limited conditions and have calculated the growth rate constant and deceleration rate constant. These data are very useful for studying the kinetic properties of bacteria.

REFERENCES

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