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DETERMINATION OF POWER –TIME CURVES OF BACTERIAL GROWTH AND STUDY OF OPTIMUM ALLOWABLE CONCENTRATION OF A SYNTHETIC MEDICINE

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Abstract

The power–time curves of *S.flexneri* 6, *S.flexneri* 3b, *S.dysenteae* and *E.coli* were determined by using the 2277 Thermal Activity Monitor (Sweden). From these curves, by using a microorganism growth experimental model with inhibitory conditions, the growth rate constant (μ) was determined at different medicinal concentrations, with the optimum allowable concentration of the synthetic medicine.

Keywords: bacterial growth, growth rate constant, microcalorimeter, optimum allowable concentration, power–time curve, synthetic medicine (Co salt)

Introduction

In any living system, the metabolic events occurring within the cells always produce reaction heat. The heat of a metabolic process in living cells can be measured by continuous measurements by using a thermal activity monitor, which is used to record the power–time curves of bacterial growth.

The power–time curves yield much information on the microorganism growth process. Information on the optimum growth temperature [1, 2], the experimental model [2, 3] and the kinetic parameters [4] may be obtained. Generally, the metabolism of bacteria is very complicated, but only a proportion of the processes have been studied.

In this paper, the power–time curves were determined under inhibitory conditions of constant temperature, volume, nutrient matter and dissolved oxygen. We have established the experimental model of bacterial growth and determined the power–time curves of *S.flexneri* 6, *S.flexneri* 3b, *S.dysenteae* and *E.coli*

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with different concentration of a synthetic medicine. We also calculated the growth rate constant and the optimum allowable concentration of the synthetic medicine.

Experimental

Instrument

A new type of heat-flow microcalorimeter, the 2277 Thermal Activity Monitor (Thermometric AB, Sweden), was used in this experiment. This system is very sensitive: the detection limit is 1.5×10^{-7} W, the baseline stability (over a period of 24 h) is 2×10^{-7} W, the range of working temperature is 10–80°C, and it can be maintained constant within $\pm2\times10^{-4}$ deg at a given temperature.

The flow–through mode was used in this experiment. The sample was pumped through the flow cell by a Micropepex pump.

Method

The procedure for the complete cleaning and sterilization of the flow tubing is as follows:

Sterilized distilled water was first pumped through the system, followed by 0.1 mol/l HCl solution, then alcoholic solution (75 vol%) and lastly again sterilized distilled water, all for 30 min at a flow rate of 30 ml/h.

Once the system had been cleaned and sterilized, distilled water was pumped through the system at a flow rate of 10 ml/h, and the baseline was determined. When a stable baseline had been obtained, the bacterial sample, the medium and the synthetic medicine were pumped into the flow cell system, and the monitor began to record the bacterial growth power–time curve of continuous growth. When the recording pen had returned to the baseline and become stabilized, the process of bacterial growth was completed.

Material

The following bacteria were employed: *S.flexneri* 6, *S.flexneri* 3b, *S.dysenteae* and *E.coli*.

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

Each 1 ml of soluble medium contained a synthetic medicine (a Co salt) at different concentrations.

The bacterial number was 10^7 cells.

Establishment of experimental model of bacterial growth

For inhibitory conditions, the bacterial number and time in the growth phase are related as follows [2, 3]:

$$dN(t)/dt = \mu N(t) - \beta N^2(t) \quad (1)$$

where μ is the growth rate constant, β is the deceleration rate constant, and $N(t)$ is the bacterial number at time t.

If the energy given out by each bacterium is P_o and the total energy at time t is $P(t)$, then

$$P(t)=P_oN(t) \quad (2)$$

Therefore,

$$dP(t)/dt=\mu P(t)-(\beta/P_o)P^2(t) \quad (3)$$

The integral of Eq. (3) is given by

$$1/P(t)=a\cdot\exp(-\mu t)+b \quad (4)$$

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

The power–time curves of the microorganism metabolic processes were determined under isothermal and isochoric condition, where the supply of the nutrient matter and oxygen was limited, and the feedback inhibitory effect of the product and the synthetic medicine (a Co salt) also existed, which is an inhibitory growth process. Strictly speaking, the exponential mode (6) could not be used to simulate the process with the inhibitory effect. Under the inhibitory condition and in the growth phase, Eq. (1) is represented well by the experimental model.

Experimental results and calculation of rate constant and optimum allowable concentration

We determined the power–time curves of (a) *S.flexneri* 6, (b) *S.flexneri* 3b, (c) *S.dysenteae* and (d) *E.coli.* at 35°C and different concentrations of inhibitor (Co salt) (see Figs 1 and 2) from the bacterial growth curves, using the $P(t)$ and t data obtained, fitted to the non-linear equation. The corresponding non-linear equation of the experimental model at 35°C and with an inhibitor concentration of 0.028 $mg \cdot ml^{-1}$
for (a) *S.flexneri* 6 is

$1/P(t)=8.7500\exp(-0.02735t)+0.0190$ for $t<200$ min with $\mu_a=0.02735$; for (b) *S.flexneri* 3b is

$1/P(t)=5.2252\exp(-0.02185t)+0.0062$ for $t<225$ min with $\mu_b=0.02185$; for (c) *S.dysenteae* is

$1/P(t)=3.0500\exp(-0.01960t)+0.05694$ for $t<200$ min with $\mu_c=0.01960$; and for (d) *E. coli* is

$1/P(t)=3.9850\exp(-0.03701t)+0.0100$ for $t<125$ min with $\mu_d=0.03701$.

In a similar way, we can also calculate the growth rate constant with different concentration of inhibitor, with the data shown in Table 1.

Table 1 Growth rate constants (μ) with different concentration (C) of inhibitor

C /mg·ml^{-1}	μ /min^{-1}			
	S.flexneri 6	*S.flexneri* 3b	*S.dysenteae*	*E.coli*
0.028	0.02735	0.02185	0.01960	0.03701
0.042	0.02027	0.01671	0.01552	
0.0504	0.01649	0.01374		
0.056	0.01441	0.01138	0.01136	0.03525
0.067	0.00889			
0.07			0.00773	
0.084				0.02555
0.098				0.01220

From these results (Table 1), we can establish the equation for *S.flexneri* 6:

$$\mu_a=0.04025-0.46769C_a \quad (R=-0.9992)$$

when $\mu_a=0$, $C_a=0.08606$ mg·ml^{-1};

for *S.flexneri* 3b:

$$\mu_b=0.03226-0.37061C_b \quad (R=-0.9997)$$

when $\mu_b=0$, $C_b=0.08706$ mg·ml^{-1};

for *S.dysenteae*:

$$\mu_c=0.02747-0.2841C_c \quad (R=-0.9996)$$

when $\mu_c=0$, $C_c=0.09670$ mg·ml^{-1};

and for *E.coli*:

$$\mu_d=0.048502-0.794595C_d+17.47704C_d^2-134.171526C_d^3$$

when $\mu_d=0$, $C_d=0.1079$ mg·ml^{-1}.

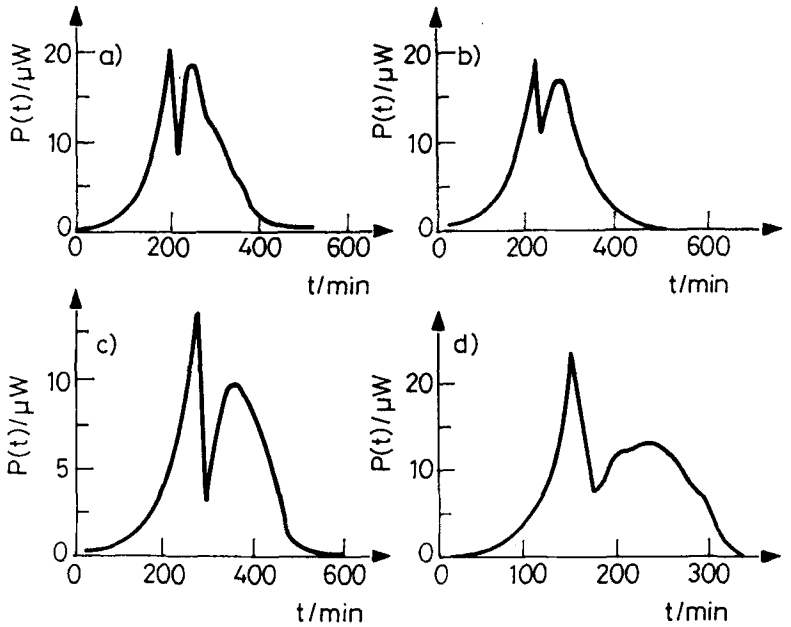

Fig. 1 Power–time curves at an inhibitor concentration of 0.028 mg/ml at 35°C. (a) *S.flexneri* 6, (b) *S.flexneri* 3b, (c) *S.dysenteae* and (d) *E.coli*

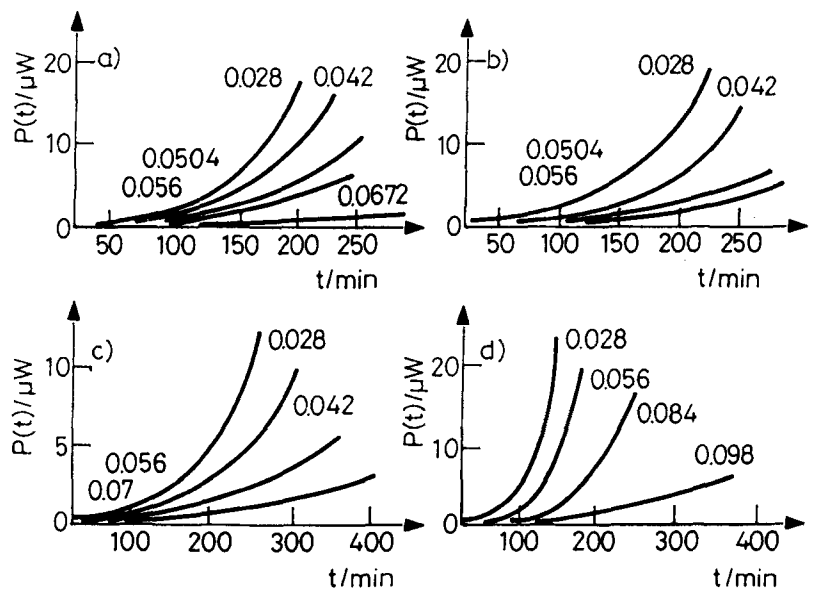

Fig. 2 Power–time curves at 35°C at different concentrations of inhibitor (Co salt) in $mg \cdot ml^{-1}$. (a) *S.flexneri* 6, (b) *S.flexneri* 3b, (c) *S.dysenteae* and (d) *E.coli*

Conclusions

Under the inhibition condition, the microorganism growth experiment model follows Eq. (1), which reflects well the actual result of the microorganism growth process.

Using the experimental model, we calculated the growth rate constant (μ) at different concentrations of the synthetic medicine, and also the optimum allowable concentration.

These are important parameters and very useful in the study of the fungistatic action of synthetic and natural medicines and the microorganism growth process.

References

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Zusammenfassung — Mittels eines Thermal Activity Monitor 2277 (Schweden) wurden die Potenz-Zeit-Kurven von *S.flexneri 6, S.flexneri 3b, S.dysenteae* und *E.coli* ermittelt. Anhand dieser Kurven wurde unter Anwendung eines experimentellen Modells für das Wachstum von Mikroorganismen unter Inhibitorbedingungen die Wachstumsgeschwindigkeitskonstante (μ) bei verschiedenen Arzneimittelkonzentrationen und in Ableitung davon die optimal erreichbare Konzentration des synthetischen Arzneimittels bestimmt.