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DETERMINATION OF POWER–TIME CURVES OF BACTERIAL GROWTH
Study of lowest growth temperature

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Abstract

Bacterial growth power–time curves were determined with a 2277 Thermal Activity Monitor. Bacterial multiplication curves were measured at different temperatures and an experimental model was established. Both growth rate constants and lowest growth temperatures were calculated.

Keywords: bacterial growth power–time curve, lowest growth temperature, microcalorimeter, rate constant

Introduction

The metabolic processes of bacteria were studied earlier by continuous calorimetry of the rates of heat production of the growing cells [1–3].

The aim of the present work was to study the rate constant and lowest growth temperature of the growth of *Escherichia coli* and *Staphylococcus albus* by means of a 2277 Thermal Activity Monitor.

Experimental

Materials

The bacterial samples used in this work were *Escherichia coli* and *Staphylococcus albus*.

A liquid medium (pH=7.2–7.4) containing NaCl (1 g), peptone (2 g) and beef extract (1 g) in 200 mL was used. The medium was filtered and autoclaved at 110°C for 20 min and stored at 5°C.

Instrument

Microcalorimetric experiments were performed on a 2277 Thermal Activity Monitor. The detection limit was 0.15 μW and the baseline stability (over a period of 24 h) was 0.2 μW. In this experiment, the stopped-flow operating mode was

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used, and the sample was pumped through the flow cells by a Microperpex pump (LKB 2132, Sweden). The performance of this instrument has been described previously [4].

Experimental

The complete cleaning and sterilization procedures for the flow tubing were as follows:

Alcohol solution (75%) and sterilized distilled water were pumped through the system for 30 min at a flow rate of 30 mL h^{-1}. Once the system had been cleaned and sterilized, the baseline was determined. After a stable baseline had been obtained, the bacterial suspension was pumped through into the flow cell at the same flow rate. When the flow cell (the volume was about 0.6 mL) was full, the pump was stopped and the monitor recorded the power–time curve of continuous bacterial growth. When the signal pen returned to the baseline, the process of bacterial growth was completed.

Calculation of growth rate constant and lowest growth temperature

According to previous papers [5, 6], the experimental equation of the bacterial growth power–time curve is

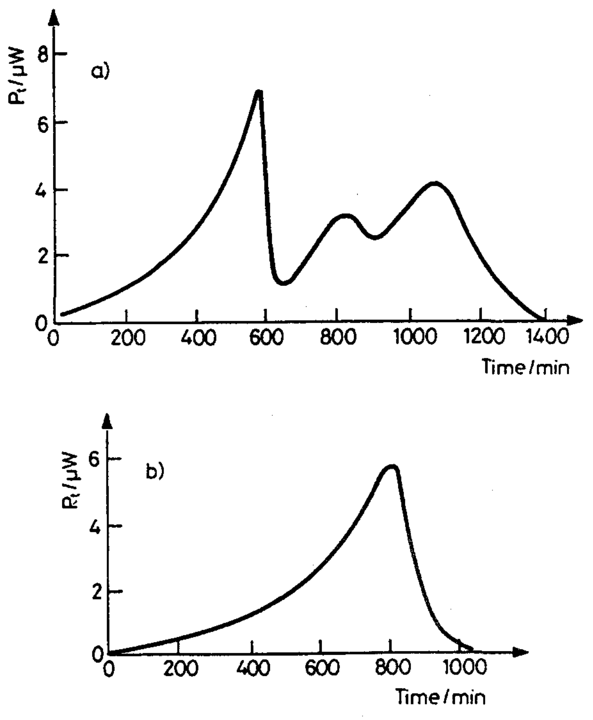

Fig. 1 Power–time curves for bacterial growth at 17°C: a) *Escherichia coli*; b) *Staphylococcus albus*. Data on *P*(*t*), *P*(*t*) and *t* are given in Table 1

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

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

Assuming that P_0 is the thermal power produced by every bacterium and $P(t)$ is the power per cell number $N(t)$, then

$$P(t) = P_0 N(t) \quad (2)$$

$$dP(t)/dt = \mu P(t) - (\beta/P_0)P^2(t) \quad (3)$$

Integration yields

$$1/P(t) = (1/p_0 - \beta/\mu P_0)\exp(-\mu t) + \beta/\mu P_0 \quad (4)$$

$$1/P(t) = (1/p_0 - \beta/\mu P_0)\exp(-\mu t) + \beta/\mu P_0 \quad (5)$$

$$\text{or } 1/P(t) = a\exp(-\mu t) + b,\ \ a = 1/p_0 - \beta/\mu P_0,\ \ b = \beta/\mu P_0 \quad (6)$$

On substitution of the data on $P(t)$ and t obtained from the bacterial growth curve into Eq. (6), the values of μ and β are obtained.

Table 1 Calorimetrically determined heat production rate $P(t)$ and calculated rate $\hat{P}(t)$ as a function of time t at 17°C

Escherichia coli			*Staphylococcus albus*		
t/min	$P(t)$/µW	$\hat{P}(t)$/µW	t/min	$P(t)$/µW	$\hat{P}(t)$/µW
50	0.4	0.40	50	0.1	0.16
100	0.5	0.52	100	0.2	0.21
150	0.7	0.69	150	0.3	0.28
200	0.9	0.90	200	0.4	0.36
250	1.1	1.18	250	0.5	0.47
300	1.5	1.55	300	0.6	0.60
350	2.0	2.04	350	0.8	0.78
400	2.8	2.68	400	1.0	1.00
450	3.5	3.52	450	1.2	1.28
500	4.6	4.61	500	1.6	1.63
550	6.1	6.05	550	1.9	2.05
575	6.8	6.92	600	2.5	2.56
			650	3.1	3.14
			700	3.8	3.82
			750	4.7	4.56
			800	5.5	5.36

Power–time curves of *Escherichia coli* and *Staphylococcus albus* at 17°C are depicted in Fig. 1.

The corresponding non-linear equation of the experimental model at 17°C for *Escherichia coli* is

$$P^{-1}(t) = 3.3109\ \exp(-0.00547t) + 0.0020 \qquad t < 575\ \text{min} \tag{7}$$

and for *Staphylococcus albus* is

$$P^{-1}(t) = 8.0025\ \exp(-0.005427t) + 0.08248 \qquad t < 800\ \text{min} \tag{8}$$

The μ *vs.* *T* (temperature) curves and the values of μ at various temperatures are shown in Table 2 and Fig. 2, respectively.

Table 2 Bacterial growth rate constants at different temperatures

T/°C	Growth rate constant μ/min	
	Escherichia coli	*Staphylococcus albus*
13	0.003227	0.002527
15	0.004246	0.004827
17	0.005470	0.005427
20	0.007886	0.009428
24	0.01386	0.01327
31	0.02227	0.02300
34	0.02660	0.03000
36		0.03970
37	0.03281	

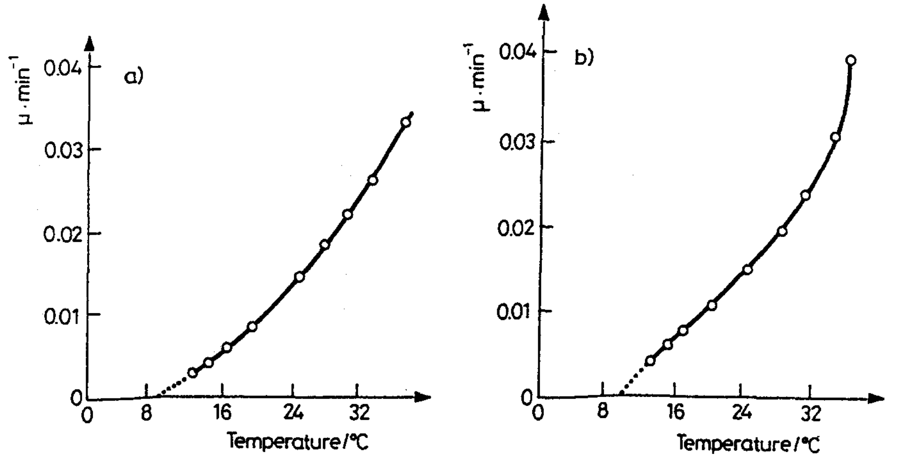

Fig. 2 Rate constant–temperature curves for bacterial growth of a) *Escherichia coli*, b) *Staphylococcus albus*

Conclusion

By substitution of the values of μ and T (from 13 to 24°C) listed in Table 2 into the linear equation $\mu = a + bT$, the following equations are obtained:
for *Escherichia coli*:

$$\mu = -0.00565428 + 6.68402 \cdot 10^{-4} T \qquad (r = 0.9942) \tag{9}$$

for *Staphylococcus albus*

$$\mu = -0.010282 + 9.7625 \cdot 10^{-4} T \qquad (r = 0.9926) \tag{10}$$

The value of T corresponding to $\mu = 0$ is known as the lowest growth temperature and is designated T_a. The values of T_a obtained for *E. coli* and *S. albus* from Eqs (9) and (10) are 8.46°C and 10.53°C, respectively. The former is similar to the 8°C reported in [6]. The latter was determined at 10.5°C. The power–time curve was always the baseline at 60 h.

The lowest growth temperature is very informative for studies on the growth of microorganisms.

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