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Measurement of multiplication rate of *Bacillus* sp. NTT-61 growth and study on its thermodynamic properties

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Abstract

The fundamental growth thermograms of *Bacillus* sp. NTT-61 have been determined by the microcalorimetric method. From these thermograms we calculate the multiplication rate constant K_a and activation energy E_a. A formula for bacterial growth is given as

$$X+S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} (X\cdot S) \xrightarrow{K_a} 2X+P$$

where X is the bacterium, S the substrate, (X·S) the bacterium–substrate complex, P the product, and K_a the rate constant. This pattern is similar to the transition state pattern in chemistry, so according to the transition state theory of reaction dynamics we can calculate the activation entropy ($\Delta S_{\neq}$) and activation free enthalpy ($\Delta G_{\neq}$) of bacterial growth processes. These are very useful data with which to study bacterial thermodynamic properties.

INTRODUCTION

Bacteria of the type *Bacillus* sp. NTT-61 are microorganisms which live in an extreme environment where the pH is above 10. In order to adapt to this environment, they have developed special structures, and physiological and biochemical functions. Therefore studies on *Bacillus* sp. NTT-61 and its possible applications in industry and agriculture are important.

We have used an LKB-2277 bioactivity monitor to determine thermograms of the growth process of *Bacillus* sp. NTT-61. The thermograms reflect the changes of bacterial growth pattern (lag phase of growth, log phase, stationary phase and the decline phase of growth). From these growth curves we are able to calculate the multiplication rate constant and activation energy of growth of *Bacillus* sp. NTT-61.

Bacterial growth is a complex series of biochemical processes. In order to use physical chemistry theory and method to study growth, we suppose that bacterial growth in culture may be divided into three steps: (1) the substrate spreads and reacts actively on the bacterium surface, (2) the bacterium absorbs the substrate and forms a bacterium · substrate complex, (3) the bacterium · substrate complex divides and produces a new bac-

terium and metabolic substance. The formula of the whole process is

$$X + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} (X \cdot S) \xrightarrow{K_a} 2X + P \tag{1}$$

where X is the bacterium, S the substrate, (X · S) the bacterium · substrate complex, P the product, and K_a is the rate constant.

According to this pattern, the first step of bacterial growth is formation of the complex (X · S), and the reaction soon reaches equilibrium

$$X + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} (X \cdot S)$$

The equilibrium constant is given by

$$K_{\neq} = \frac{[X \cdot S]}{[X][S]}$$

In the second step, the complex (X · S) splits to produce a new bacterium and give a metabolic substance. This step is the slowest in the whole process, and so controls the rate of bacterial growth.

The pattern of bacterial growth is similar to transition state theory of a chemical process, so

$$K_a = \frac{kT}{h} \exp(\Delta S_{\neq}/R) \cdot \exp(-E_a/RT) \tag{2}$$

$$\Delta G_{\neq} = \Delta H_{\neq} - T\,\Delta S_{\neq} = E_a - T\,\Delta S_{\neq} \tag{3}$$

$$K_{\neq} = \exp(-\Delta G_{\neq}/RT) \tag{4}$$

If values of K_a and E_a have been determined, activation entropy $\Delta S_{\neq}$, activation free enthalpy $\Delta G_{\neq}$ and equilibrium constant $K_{\neq}$ in the first step can be calculated.

INSTRUMENT AND MATERIALS

Instrument

An LKB-2277 bioactivity monitor was used to determine the thermograms of bacterial growth. The performance of this instrument and the details of its construction have been described in refs. 1 and 2. In this experiment the flow through method is used, and the schematic diagram of cycle flow cell is shown in Fig. 1.

Materials

The bacterial strain *Bacillus* sp. NTT-61 (alkalophilic bacterium) was used which can grow well up to pH 10.

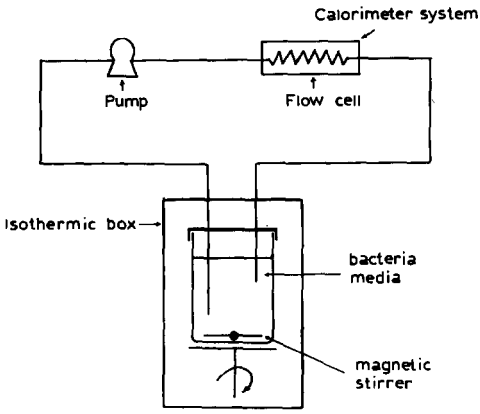

Fig. 1. Schematic diagram of bacterial culture in cycle flow cell.

A soluble starch medium (pH 10) was used, containing per 1000 ml the following: soluble starch, 10 g; $(NH_4)_2SO_4$, 2 g; yeast extract, 2.5 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $NaCO_3$ (10%), applicable amount.

EXPERIMENT AND RESULTS

Experiment

The experimental set-up is shown in Fig. 1. Firstly the flow cell was cleaned and sterilized, using 0.1 N H_2SO_4 and 75% alcohol solution. Then sterilized distilled water was pumped through the system at a flow rate of 10 ml h^{-1} to run the baseline. After a stable baseline had been obtained, the bacterial sample was pumped into the flow cell system by the cyclic flow method, and the monitor recorded the thermogram of bacterial growth continuously. When the recording pen returned to the baseline and became stabilized the process of bacterial growth was complete.

Results

A thermogram of *Bacillus* sp. NTT-61 growth is shown in Fig. 2. The four phases of growth discernible are lag phase, log phase, stationary phase and decline phase. From the log phase of the growth curve the multiplication rate constant of *Bacillus* sp. NTT-61 can be calculated.

Calculation of the multiplication rate constant

In the log phase of growth, bacterial numbers and culture time are in accordance with an exponential law, so that

$$n_t = n_0 \exp(Kt)$$

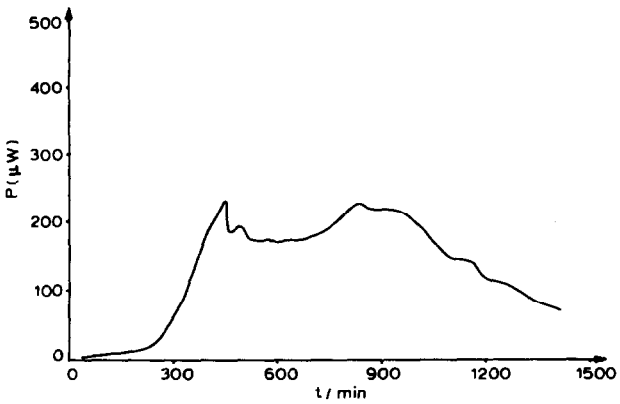

Fig. 2. Thermogram of *Bacillus* sp. NTT-61 obtained at 37 °C.

If the power given out by every bacterium is w, then

$$P_0 = n_0 w \qquad P_t = n_t w$$

so we have

$$P_t = P_0 \exp(Kt)$$

or

$$\ln P_t = ln\, P_0 + Kt$$

Using the data ($\ln P_t$ and t) obtained from the bacterial culture curve to fit a linear equation, one can obtain the rate constant K. Data for P_t, t and the rate constant K are shown in Table 1.

TABLE 1

P_t and t values for *Bacillus* sp. NTT-61 at 37 °C [a]

t (min)	Expt. no. 1		Expt. no. 2	
	P_t (μW)	$\ln P_t$	P_t (μW)	$\ln P_t$
50	2.3	0.819	1.8	0.597
100	6.8	1.918	4.3	1.462
150	9.1	2.206	6.8	1.918
200	12.5	2.525	13.6	2.612
250	19.3	2.960	29.5	3.380
300	51.1	3.933	65.8	4.187
350	105.6	4.659	131.7	4.880
400	180.5	5.196	217.9	5.384
450	299.6	5.706		

[a] $\ln P_t = 0.3499 + 0.01189t$, $r = 0.991$, $K_1 = 0.01189$ (min^{-1}); $\ln P_t = 0.6396 + 0.01385t$, $r = 0.998$, $K_2 = 0.01385$ (min^{-1}); average $K_a = 0.01287$ (min^{-1}).

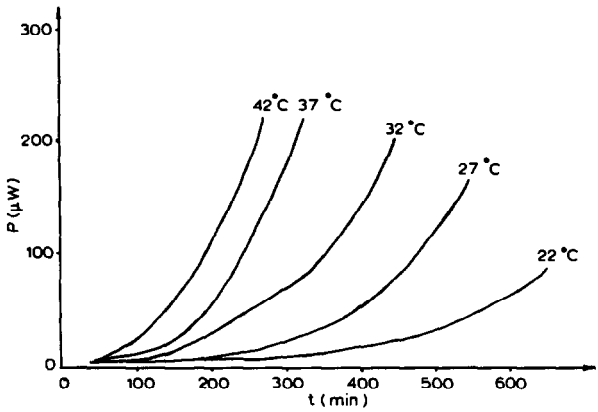

Fig. 3. Thermograms of *Bacillus* sp. NTT-61 growth in log phase at different temperatures.

Activation energy of bacterial growth

The thermograms of *Bacillus* sp. NTT-61 at different temperatures have been determined using the same experimental method; the rate constants (K) at each temperature were calculated and the results indicate that ln K and $1/T$ are related linearly so with the help of the Arrhenius relation, the activation energy can be calculated. Thermograms of growth at different temperatures are shown in Fig. 3. and rate constants are displayed in Table 2.

Activation entropy and activation free enthalpy of bacterial growth

Values obtained from the growth curves at various temperatures K_a and E_a values determined experimentally have been substituted in eqns. (2)–(4) to give activation entropy ($\Delta S_{\neq}$) and activation free enthalpy ($\Delta G_{\neq}$) values. These data are shown in Table 3.

TABLE 2

Rate constants of NTT-61 growth at various temperatures [a]

T (°C)	K (min^{-1})	K (s^{-1})
22	0.006123	0.0001020
27	0.007566	0.0001261
32	0.009277	0.0001546
37	0.012870	0.0002145
42	0.014000	0.0002333

[a] Activation energy $E_a = 33.8$ kJ mol^{-1}; correlation coefficient $r = 0.99$.

TABLE 3

Values of $\Delta S_{\neq}$, $\Delta G_{\neq}$ and $K_{\neq}$

	T (°C)				
	22	27	32	37	42
$\Delta S_{\neq}$ (J kmol^{-1})	−206.6	−206.9	−207.2	−206.8	−207.9
$\Delta G_{\neq}$ (kJ mol^{-1})	94.75	95.87	96.99	97.91	99.29
$K_{\neq}$ ($\times 10^{17}$)	1.668	2.027	2.448	3.174	4.425

CONCLUSION

The thermogram of *Bacillus* sp. NTT-61 can be determined by a microcalorimetric method. These thermograms contain much information concerning the kinetics of metabolic processes and from them we can measure the multiplication rate constant (K) and activation energy (E_a) of bacterial growth.

The pattern of bacterial growth, $X + S \rightleftharpoons (X \cdot S) \rightarrow 2X + P$ enables the activation entropy ($\Delta S_{\neq}$) and activation free enthalpy ($\Delta G_{\neq}$) to be calculated; these data are very useful for studying bacterial thermodynamic properties.

The entropy ($\Delta S_{\neq}$) values of these experiments are all below zero. According to thermodynamics, a large decrease in entropy in a reaction means that products are more orderly than reactants. Thus absorption of substrate by the bacterium and formation of the bacterium · substrate complex being a process of entropy decrease is a change in which the system goes from less orderly to more orderly.

ACKNOWLEDGEMENT

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