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DETERMINATION OF POWER–TIME CURVES OF BACTERIAL GROWTH AND STUDY OF OPTIMUM GROWTH TEMPERATURE AND ACIDITY

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Abstract

Bacterial growth power–time curves were determined by using the 2277 Thermal Activity Monitor. The growth rate constants at different temperatures and acidities were calculated via the optimum growth temperature and optimum growth acidity obtained.

Keywords: bacterial growth, optimum growth acidity, optimum growth temperature, power–time curve

Introduction

In two previous papers [1, 2], we have reported on the determination of power–time curves for bacterial growth, study of the optimum growth temperature, and the establishment of an experimental model of bacterial growth.

In the present paper, we have again used a 2277 Thermal Activity Monitor to determine the power–time curves of *Staphylococcus albus (S.albus)* and *Escherichia coli (E.coli)* at different temperatures. From these growth curves, we can calculate the growth rate constant. We obtained the temperatures T_o of optimum growth. At the temperatures T_o, we determined the power–time curves of the two kinds of bacteria at different acidities. We additionally obtained the acidity pH_o of optimum bacterial growth.

In [1], we obtained the optimum temperature by using the experimental law. In this paper, we have used the experimental law of inhibition condition [2].

In [3], G. Liden *et al.* studied the change in acidity in the metabolism of microorganisms. A change in acidity must affect bacterial growth. In this paper, we used a buffer solution. In the metabolism of the bacteria, the acidity was approximately constant.

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Experimental

Instruments

1) A new type of microlcalorimeter, a 2277 Thermal Activity Monitor (Sweden), was used in these experiments. With this instrument, reactions can be studied in the temperature range 10–80°C (the working temperature range of the thermostat). It was maintained at a temperature within $\pm 2\times 10^{-4}$ deg. The detection limit was 0.15 μW, and the baseline stability (over a period of 24 h) was 0.2 μW.

In these experiments, the flow-through mode of the thermal activity monitor was used. The sample was pumped through the flow cell of the calorimeter by a microperpex pump.

2) A glass electrode *pH*-meter was used (mode HM-20S, TOA Electronics Ltd, Japan) with a range of pH=0.00–14.

Method

The complete cleaning and sterilization procedure for the flow tube was as follows:

1) Sterilized distilled water was pumped through the system for 30 min at a flow rate of 30 ml/h.

2) An alcohol solution (75 vol%) was pumped through the system for 30 min at a flow rate of 30 ml/h.

3) Sterilized distilled water was again pumped through the system for 30 min at a flow rate of 10 ml/h and the baseline was determined.

When a stable baseline was obtained, the bacterial sample was pumped into the flow cell system and the monitor began to record the power-time curves.

Materials

The bacteria employed were *E.coli* and *S.albus*.

A) Liquid medium (pH=7.3) containing NaCl (1 g), peptone (2 g) and beef extract (1 g) per 200 ml water was used.

B) Liquid medium (pH=5.00–10.00) containing NaCl (1 g), peptone (2 g), beef extract (1 g), H_3PO_4 (0.784 g,85 vol%), CH_3COOH (0.48 g), H_3BO_3 (0.49 g) and NaOH in different amounts per 200 ml water was used.

The buffer solution consisted of H_3PO_4 (0.784 g, 85 vol%), CH_3COOH (0.48 g), H_3BO_3 (0.49 g) and NaOH in different amounts per 200 ml water [4].

Theoretical

Under limited growing conditions, a model of bacterial growth was given by the following Eq. [5]:

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

where μ is the growth rate constant;

β is the deceleration rate constant;

$N(t)$ is the number of bacteria at time t.

Under the assumption that the heat production P_t is proportional to the number of bacteria N, and P_o is the heat production of one cell [2]:

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

Eq. (1) transforms to

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

The integral Eq. (3) is given by

$$1/P(t) = (1/P_o - \beta/\mu P_o)e^{-\mu t} + \beta/\mu P_o \quad (4)$$

or $1/P(t) = ae^{-\mu t} + b$

with $a = 1/P_o - \beta/\mu P_o \quad b = \beta/P_o$

Using the experimental data $P(t)$ and t obtained from the bacterial growth curves and fitting them to the non-linear equations, we can obtain the growth rate constant (μ).

From the rate constants at different temperatures, a non-linear equation of the form $\mu=aT^3+bT^2+cT+d$ can be established and the optimum growth temperature (T_o) can be calculated.

At this optimum growth temperature (T_o), we studied the power–time curves of bacterial growth at different acid concentrations and calculated the growth rate constant. From these rate constants at different acidities, we also established a non-linear equation $\mu=a+bpH+cpH^2+dpH^3$ and calculated the optimum growth acidity (pH_o) of bacterial growth.

Results

We determined the power–time curves of *E.coli* and *S.albus* at the growth temperature in the medium (A), and calculated the growth rate constants at various temperatures. These data are shown in Table 1.

From these results, the non-linear equation $\mu=aT^3+bT^2+cT+d$ can be established. The maximum in the growth rate constant μ leads to the T_o of optimum growth temperature.

Table 1 Growth rate constants μ of bacteria in medium (A) at different temperatures

Temp. /K	304	307	309	310	311	313
S. albus	0.0230	0.0300		0.0351		0.0310
μ /min	(0.0230)	(0.0300)		(0.0351)		(0.0310)
E. coli		0.0266	0.0397		0.0386	0.0355
μ /min		(0.0266)	(0.0391)		(0.0386)	(0.0355)

( ): data calculated from Eqs (5) and (6)

For *S. albus*:

$$\mu=1278.69008-12.5332246T+0.0409413087T^2-4.45710158\times10^{-5}T^3 \quad (5)$$

$T_o=310.52$ K

For *E. coli*:

$$\mu=-7612.63044+73.3587075T-0.235631034T^2+0.000252777991T^3 \quad (6)$$

$T_o=309.66$ K

Table 2 Growth rate constants μ of bacteria at various acidities

pH	6.28	6.92	7.26	7.91	8.27	8.80
S. albus	0.008800	0.01156	0.01144	0.008054	0.005697	0.002465
μ /min	(0.008783)	(0.01166)	(0.01123)	(0.008077)	(0.005715)	(0.002455)
E. coli	0.01370	0.01530	0.01610	0.01680	0.01600	0.01410
μ /min	(0.01368)	(0.01536)	(0.01609)	(0.01664)	(0.01617)	(0.01406)

( ): data calculated from Eqs (7) and (8)

Moreover, we studied the growth rate constant at the optimum growth temperature T_o in medium (B) and different acidities. These data are shown in Table 2. Graphs may be seen in Figs 1 and 2.
From these results, we can establish a non-linear equation of the form

$$\mu = a + bpH + cpH^2 + dpH^3$$

The maximum in μ leads to the optimum growth acidity pH_o.

For *S. albus*:

$$\mu = -0.719025448 + 0.278082733pH - 0.0346549976pH^2 + 0.00140583242pH^3 \quad (7)$$

$pH_o = 6.96$

For *E. coli*:

$$\mu = 0.129472 - 0.0594593206pH + 0.00968688045pH^2 - 0.000502313pH^3 \quad (8)$$

$pH_o = 7.79$

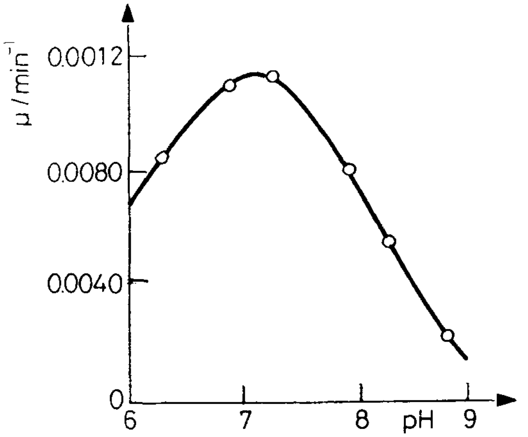

Fig. 1 μ *vs. pH* curve for *S. albus* at 310.52 K

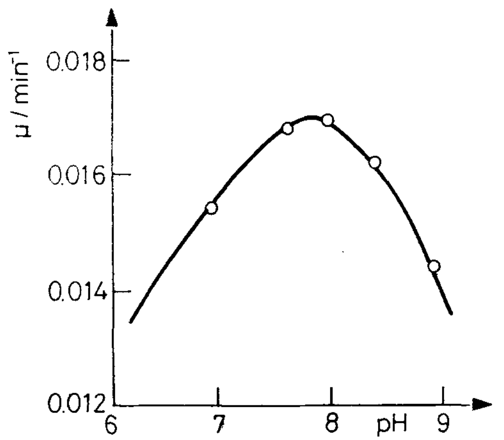

Fig. 2 μ *vs. pH* curve for *E. coli* at 309.66 K

Conclusions

Optimum growth temperatures of 310.52 and 309.66 K have been obtained for *S.albus* and *E.coli* by using a model described earlier [2]. At the optimum growth temperature, we obtained a non-linear equation for μ as a function of *pH* at various acidities in medium (B). From these, the optimum growth acidity was obtained: for *S.albus* pH_o=6.96, and for *E.coli* pH_o=7.79.

These obtained data (T_o and pH_o) are very useful theoretically and practically for study of cultivation and inhibition of bacterial growth.

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Zusammenfassung — Mittels eines Thermal Activity Monitors 2277 wurden Potenz-Zeit-Kurven bakteriellen Wachstums ermittelt. Anhand der ermittelten optimalen Wachstumstemperatur und Wachstumsazidität wurden die Wachstumsgeschwindigkeitskonstanten für verschiedene Temperaturen und Aziditäten berechnet.