Thermochimica Acta, 158 (1990) 187–193
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THERMOCHEMICAL STUDIES ON CELL METABOLISM. PART I. THERMAL EQUATIONS FOR THE METABOLISM OF RESTING CELLS

XIE CHANG-LI, SUN DAY-UNG, SONG ZHAU-HUA and QU SONG-SHENG
Department of Chemistry, Wuhan University, Wuhan (China)

LIAO YAO-TING and LIU HAI-SHUI
Army Hospital of Kwangchow Military District, Wuhan (China)

(Received 11 July 1989)

ABSTRACT

The heat output of the non-growth metabolism of resting cells of *Escherichia coli* has been determined using an LKB-2277 bioactivity monitor. The experimental results indicate that there is a linear relationship between the metabolic power and the cell concentration and that the heat output produced by a single cell's metabolism depends on the cell concentration and is inhibited by the cell density.

INTRODUCTION

The various metabolic events occurring within cells are all heat-producing reactions. Thus, by monitoring the heat effects with sufficiently sensitive calorimeters, the metabolic processes of living cells can be studied. In general, the metabolism of cells (as bacteria) is very complicated. To facilitate our research, we have studied the classic metabolic processes of bacteria: the growth metabolism, the endogenous metabolism and the non-growth metabolism of resting cells, respectively. In the present work, an LKB-2277 bioactivity monitor has been used to determine the heat output of the non-growth metabolism of resting cells of *Escherichia coli*. The experimental results indicate that the relationship between cell concentration and their heat output can be characterised by the following equations

$$C = kP + a \tag{1}$$

$$\mathrm{d}C/\mathrm{d}P_0 = KC^2 \tag{2}$$

where P is the heat output (μW), C is the cell concentration (mg ml^{-1}), P_0 is the heat output produced by a single cell's metabolism, $P_0 = P/C$, and k, a and K are constants which depend on the cultural condition of the cells

0040-6031/90/$03.50

such as temperature and medium. Equations (1) and (2) indicate that the metabolism power has a linear relationship with the cell concentration and that the metabolism of a single cell, P_0, depends on the cell concentration and is inhibited by the cell density (the number of cells, or dry weight, per unit volume of medium).

INSTRUMENT AND MATERIALS

An LKB-2277 bioactivity monitor was used to determine the metabolic power of the cells. The performance of this instrument and the details of its construction have been previously described [1,2].

Materials

The materials used, including the *E. coli* (ACCT), were provided by the Army Hospital of the Kwangchow Military Area.

Peptone medium

1000 ml of the peptone medium contained the following: NaCl, 5 g; K_2HPO_4, 2 g; *p*-aminobenzoic acid, 0.02 g; peptone, 5 g; sodium citrate, 12 g; $MgSO_4$, 5 g; tryptone, 5 g; beef extract, 3 g; tryptose, 5 g; and NaOH (2 N), 2.5 ml.

Buffer (pH 7)

1000 ml of buffer contained the following: 0.2 M KH_2PO_4, 66 ml; 0.2 M K_2HPO_4, 144.5 ml; NaCl, 0.5 g; $MgSO_4$, 0.12 g; and a trace of $CaCl_2$ and $FeCl_2$.

Glucose media

Glucose medium A contained 2 g of glucose and 1 g of NH_4Cl in 1000 ml of buffer. Glucose medium B, for non-growth culture, contained 2400 μg of glucose per ml of buffer solution.

EXPERIMENTAL METHOD

Preparation of the resting cells

The *E. coli* stain was first inoculated into the peptone medium or into glucose medium A. When the cells go into log phase, 10 ml of the bacterial sample were removed and separated in a centrifuge (10^4 rpm); the residual medium was then washed away with buffer solution. Finally, these bacterial cells were suspended in 25 ml of buffer solution containing 80 μg ml^{-1} glucose. This bacterial sample was pumped into the flow cell and the

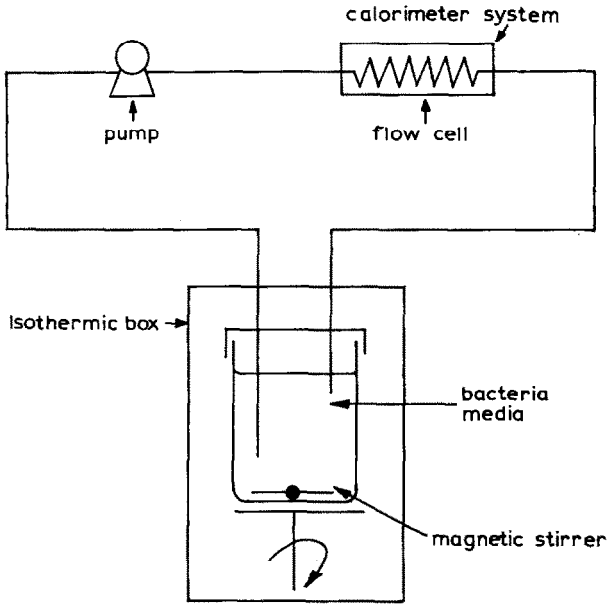

Fig. 1. Schematic diagram of cycle flow cell.

thermogenesis curves were monitored by the cycle-flow method (temperature at 37 °C). A schematic representation of the experimental apparatus is shown in Fig. 1. The return of the chart-recorder pen to the baseline indicates that the cells are going into the resting state [3]. These resting cells were then used for the non-growth metabolism experiment.

Experimental determination

Glucose medium B (5 ml) was added to the samples of resting cells such that the total volume was 30 ml. These resting cells were used to study the

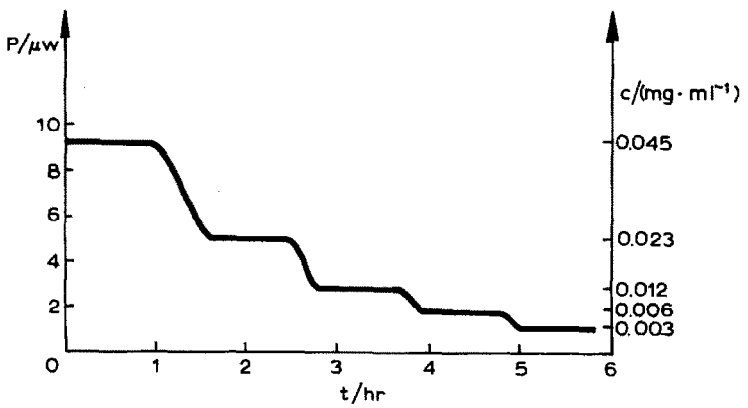

Fig. 2. Thermogenesis curve of the resting metabolism of *E. coli* (obtained from beef medium).

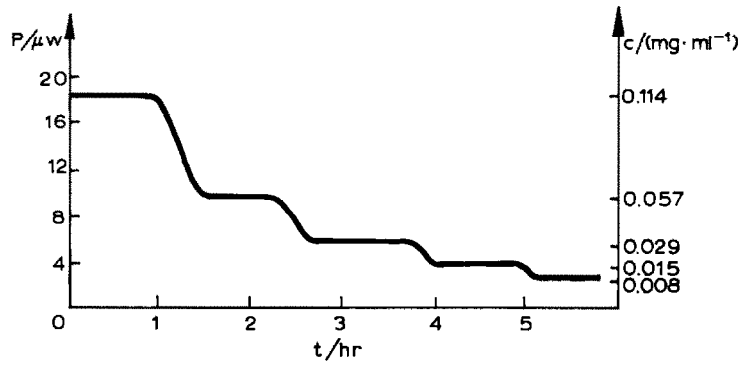

Fig. 3. Thermogenesis curve of the resting metabolism of *E. coli* (obtained from glucose medium).

non-growth metabolism, the metabolic power being determined by the cycle-flow method. When the metabolic power output was steady, indicated by a plateau on the thermogenesis curve, 15 ml of the bacterial sample removed and 15 ml of glucose medium (B) were added, thereby reducing the cell concentration by half. Continuing to monitor using the cycle-flow method reveals further plateaus in the thermogenesis curve. The above operation was repeated 4–5 times until the power output plateau approached the baseline. The experimental curves are shown in Figs. 2 and 3.

In order to measure the cell concentration, three 10 ml bacterial samples were removed from the culture medium, separately centrifuged, then washed with distilled water to remove the residual medium, heated and weighed. The average dry weight of the cells (mg ml)$^{-1}$) can then be calculated.

RESULTS

The C–P data for the metabolism of the resting cells of *E. coli* from peptone medium and glucose medium were obtained from the thermogenesis curves, see Figs. 2 and 3, respectively. The data are presented in Tables 1 and 2.

The C–P data given in Table 1 indicate that C and P are linearly proportional (see Fig. 4): the corresponding linear equation is $C = 0.005\,267P - 0.003\,595$, with correlation coefficient r 0.9999.

Using the $1/C$ and P_0 values from Table 1 to fit a linear equation (see Fig. 5), another equation can be obtained as follows: $1/C = 0.8605P_0 - 270.26$, with $r = 0.9998$.

Similarly, from the data in Table 2, the corresponding linear equations, see Figs. 6 and 7, can be obtained: $C = 0.006\,908P - 0.011\,35$, $r = 0.9998$; and $1/C = 0.3452P_0 - 81.52$, $r = 0.9998$.

TABLE 1

$C-P$ data for *E. coli* resting metabolism (obtained from beef medium)

	Experiment no.				
	1	2	3	4	5
C (mg ml^{-1})	0.0451	0.0228	0.0114	0.0057	0.0029
P (μW)	9.25	5.00	2.85	1.75	1.25
P_0 (μW per mg ml^{-1}) [a]	341.9	365.6	416.7	512.3	717.2
$1/C$ (ml mg^{-1})	22.17	43.86	87.72	175.4	344.8

[a] $P_a = P/(0.6C)$ (flow cell volume $V = 0.6$ ml).

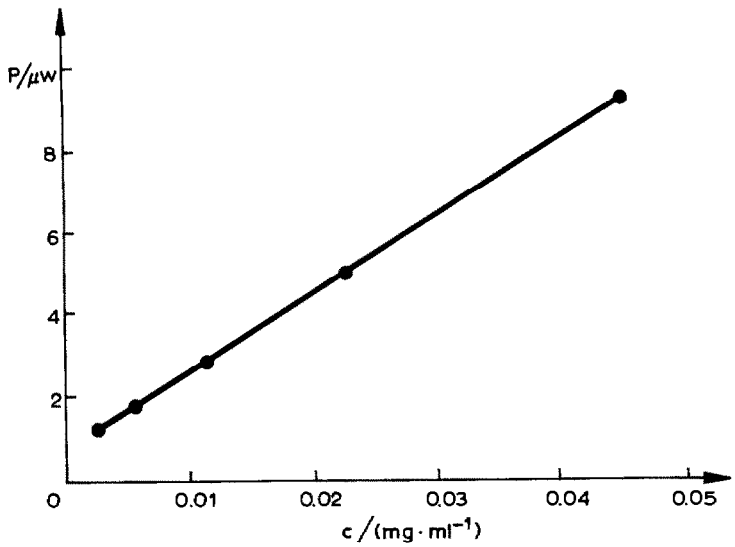

Fig. 4. $C-P$ relation for the resting metabolism of *E. coli* (obtained from beef medium).

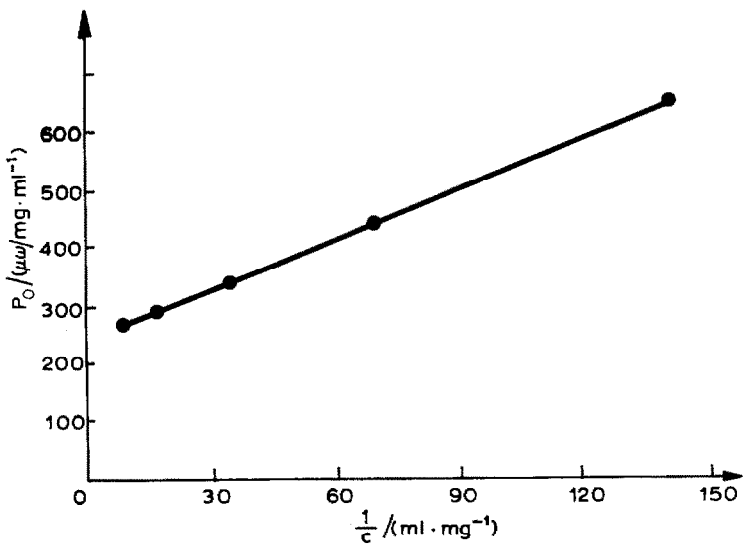

Fig. 5. $1/C-P_0$ relation for the resting metabolism of *E. coli* (obtained from beef medium).

TABLE 2

$C-P$ data for *E. coli* resting metabolism (obtained from glucose medium)

	Exponent no.				
	1	2	3	4	5
C (mg ml^{-1})	0.1142	0.0571	0.0286	0.0143	0.0071
P (μW)	18.25	9.75	5.75	3.75	2.75
P_0 (μW per mg ml^{-1}) [a]	266.4	284.6	335.2	437.2	645.7
$1/C$ (ml mg^{-1})	8.76	17.51	34.97	69.93	140.95

[a] $P_0 = P/(0.6C)$ (flow cell volume $V = 0.6$ ml).

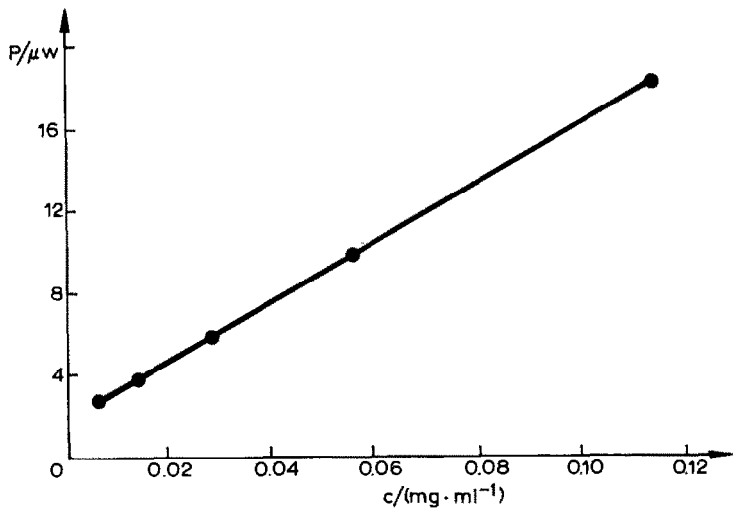

Fig. 6. $C-P$ relation for the resting metabolism of *E. coli* (obtained from glucose medium).

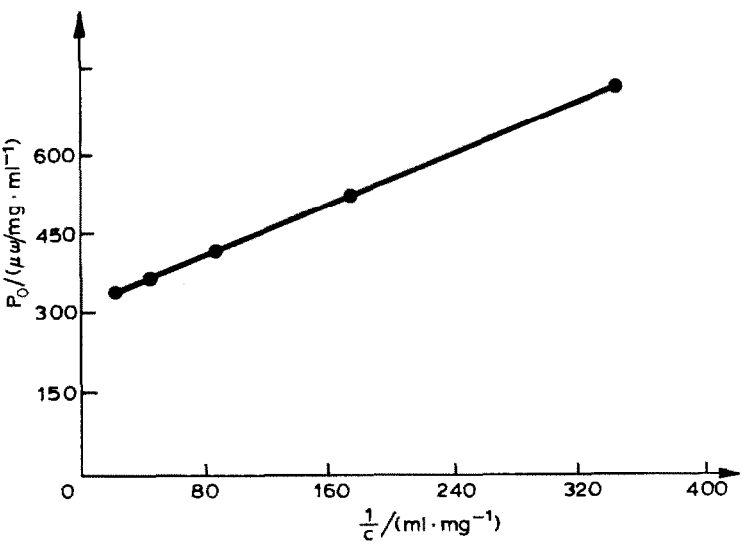

Fig. 7. $1/C-P_0$ relation for the resting metabolism of *E. coli* (obtained from glucose medium).

CONCLUSIONS

The heat output of the non-growth metabolism of resting cells of *E. coli* has been determined. The experimental results indicate that the relationship between cell concentration and heat output can be characterised by the equations

$$C = kP + a \quad \text{and} \quad 1/C = KP_0 + A \qquad \text{or} \qquad C = kP + a \quad \text{and} \quad dC/dP_0 = KC^N \quad \text{(for resting cells, } N = 2\text{)}$$

These equations indicate that the metabolic power and the cell concentration are in linear relation, and that P_0 (the single cell metabolic power) depends on the cell concentration, and is inhibited by the cell density, suggesting the presence of a space effect.

The experimental results confirmed the applicability of the equations, with the correlation coefficient r being greater than 0.99 although the *E. coli* resting cells were obtained from different culture media. In general, different cultural conditions only change the values of the constants (k, a and K) of the equation. Thus, these equations specifically characterise the metabolic process of the resting cells and provide a functional relationship for the metabolism of the resting cells.

REFERENCES

1 C.-L. Xie, H.-K. Tang, Z.-H. Song and S.-S. Qu, Thermochim. Acta, 123 (1988) 33.
2 J. Suurkuusk and I. Wadso, Chem. Scr., 20 (1982) 155.
3 D.-U. Sun, H.-K. Tang, C.-L. Xie and S.-S. Qu, Acta Physicochim. Sin., 3 (5) (1987) 465.