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THE EFFECT OF ZINC(II) ON THE GROWTH OF *E . COLI* STUDIED BY MICROCALORIMETRY

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By using an LKB2277 Bioactivity Monitor, stop-flow mode, the power–time curves of *E . coli* at 37°C effected by zinc(Ⅱ) were determined. Some parameters, such as growth rate constants k, inhibitory ratio I, the maximum heat production rate P_{max} heat output Q and the time in the maximum heat production t_{max} were obtained. According these parameters, we found that a low concentration of zinc(Ⅱ) had a promoting action on the growth of *E. coli,* but a high concentration of zinc(Ⅱ) had an inhibitory action. The toxicity of zinc(Ⅱ) can also be expressed as half inhibitory concentration IC_{50} of zinc(Ⅱ), *i.e*., 50% effective in this inhibition. The value of IC_{50} of zinc(Ⅱ) on *E. coli* is 28.09 μg mL^{-1}. The assay is quantitative, inexpensive and versatile.

Keywords: *E . coli, effect of zinc(II), metabolic action, microcalorimetry*

Introduction

Zinc is one important trace element in living body. The amount of zinc in organism body is second to the Fe. Moreover, among the six enzyme, oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases, zinc–enzyme exists everywhere. Until now, from the different species, about 300 kinds of zinc-protein have been found, which act as more than 20 kinds of functions, it almost refers to any aspects of metabolism, including ability [1, 2]. Zinc breaking the balance of metabolism may cause many diseases.

Microcalorimetry is a very useful method that can be applied to study microbial growth. Many calorimetric studies have been made on microbial systems in order to obtain quantitative information about their biochemical and physiological activities [3–6]. But calorimetry has seldom been applied to study the effects of trace element on the growth of microbial. Microcalorimetry has the advantage that the method is specific only to the initial and final energy states of a system and that it is independent of the organisms or the reaction pathway. The heat output is derived largely form the catabolic breakdown of substrate, anabolic reaction contributing little to the overall balance [7–9].

In this work, we have used microcalorimetry to study the effect of zinc(II) on the growth of *E. coli* and its relationship with the rate of heat production and microbial growth so as to provide useful information for production.

Experimental

Materials

E. coli (CCTCC HB101) was provided by China Center of type Culture Collection, Wuhan University, Wuhan 430070, P. R. China.

$ZnCl_2$ (analytical grade) as supplied by the Shanghai Yuelong Chemical Factory, Shanghai, P. R. China.

The peptone culture medium was a solution contained per 1000 mL (pH=7.0): peptone 5 g, beef extract 3 g and NaCl 5 g. It was sterilized in high-pressure steam at 120°C for 30 min.

Instrument

A microcalorimeter, LKB-2277 BioActivity Monitor manufactured by LKB corporation of Sweden was used to determine the growth metabolic power-time curves of *E. coli* cells. The microcalorimeter was thermostated at 37°C. The voltage signal was recorded by means of an LKB-2210 recorder (1000-mV range). The baseline stability was 0.2 W/24 h. The performance of this instrument and the details of its construction have been previously described [10, 11].

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Preparation of the sample

Initially, *E. coli* was inoculated in the prepared peptone culture medium, containing $2 \cdot 10^6$ cells mL^{-1}. The cells were suspended in the peptone culture medium, then zinc(II) of different concentration 0, 1, 2, 5, 10, 20, 30, 40, 50 μg mL^{-1} were added into the culture medium at once. These solutions were prepared freshly.

Methods

In the calorimetric experiment, the flow call was completely cleaned and sterilized. The procedure was: Sterilized distilled water including 0.1 mol L^{-1} NaOH, 75% alcohol solution and 0.1 mol L^{-1} HCI were pumped in sequence by a LKB-2132 microperplex peristaltic pump through the cell, each for 15 min at a flow rate of 50% mL h^{-1}.

Once the system was cleaned and sterilized and the baseline was stabilized, the bacterial suspension, initially containing $2 \cdot 10^6$ bacteria mL^{-1} and zinc(II) was pumped through the calorimetric cell with an LKB-2132 perplex peristaltic pump at a low rate of 50 mL h^{-1}. When the flow cell (volume 0.6 mL) was full, the pump was stopped and the monitor was used to record the power–time curves of the bacterial growth.

In this type of experiment, the bacteria used were suspended in the peptone culture medium, then zinc(II) of different concentration 0,1, 2, 5, 10, 20, 30, 40, 50 μg mL^{-1} were added into the culture medium. These solutions were prepared freshly.

Results and discussion

Power-time curves

Figure 1 shows the power–time curves obtained when a culture of the bacteria was inoculated zinc(II) at different concentrations. During 5–50 μg mL^{-1} , with increasing of concentration of zinc(II), the log phase became longer, the maximum power decreased. These results suggested that zinc(II) could inhibit the bacterial growth. While during 0–2 μg mL^{-1}, contrary to the above one, it indicated that a low concentration of zinc(II) had a promoting action on the growth of *E. coli.*

Thermokinetics

In the log phase of growth, the power–time curve obeys the equation:

$$\ln P_t = \ln P_0 + kt \quad (1)$$

Using this equation, the growth rate constants k of all experiments were calculated and the concentration times (G), which equal (ln2)/k, were also obtained. The corresponding between k and G are shown in Table 1.

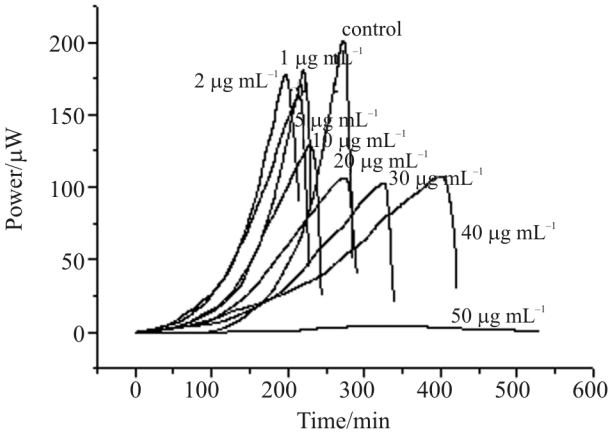

Fig. 1 The power-time curve for the growth of *E. coli* at different concentrations of zinc(II) at 37°C

Relationship between k and c

The data in the Table 1 shows that the growth rate constant k changed with an increase in the mass of zinc(II). The k–c equations can be obtained:

k=0.02508+0.00119c R=0.9866 (0–2 μg mL^{-1})

k=0.02652–3.6427·$10^{-1}c$ R=0.9908 (5–50 μg mL^{-1})

Inhibitory ratios and half inhibitory concentrations

High concentrations of zinc(II) will inhibit *E. coli* growth, the inhibitory ratio I is defined as:

$$I=[(k_0-k_c)/k_0]\cdot 100\% \quad (2)$$

where k_0 is the rate constant of the control, and k_c is the rate constant for *E. coli* growth inhibited by an inhibitor whose concentration is c. When the inhibitory ratio I is 50%, the corresponding concentration of inhibitor is called as the half inhibitory IC_{50}. According to the k~c equations, the value of IC_{50} can be obtained. The values of I and IC_{50} are also shown in Table 1.

The relationship of P_{max} and c

From Table 1, during 2–50 μg mL^{-1}, we can see the maximum heat power of growth phase P_{max} decreased with the increasing of zinc(II) concentration, it was not linear between P_{max} and c.

The relationship of Q_{lag} and c

From the data in Table 1, if we made fit linear between Q_{lag} and c, then we can obtaine the linear relationship of Q_{lag} and c. It shows a very good linear relationship.

Q_{lag} =0.4779+0.00703c, R= –0.9863 (5~50 μg mL^{-1}).

Table 1 The thermokinetic equation for *E. coli* growth at different concentration of zinc(II) at 37°C

c/μg mL^{-1}	ln$P$~t	k/min^{-1}	R
0	ln$P$ =0.9143+0.02518t	0.02518	0.9924
1	ln$P$ =2.39339+0.02607t	0.02607	0.9921
2	lnP=0.54002+0.02756t	0.02756	0.9940
5	ln$P$=0.03845+0.02489t	0.02489	0.9915
10	ln$P$=2.9194 +0.02237t	0.02237	0.9903
20	ln$P$=2.80689+0.01925t	0.01925	0.9869
30	ln$P$=2.39405+0.01641t	0.01641	0.9893
40	ln$P$=2.93887+0.01143t	0.01143	0.9972
50	–	–	–

The relationship of Q_{lag} and k

From the data in Table 1, it also shows linear relationship of Q and k during 5~50 μg mL^{-1}.

Q_{lag} =0.97863–18.7508k , R= –0.9906 (5~50 μg mL^{-1}).

The relationship of t_{max} and c

From the data in Table 1, we can see that with the increasing of zinc(II) concentration during 5–40 μg mL^{-1}, the growth of *E. coli* was inhibited, the maximum time of the growth phase (t_{max}) increased. If we fit linear relationship between t_{max} and c, then we can obtaine the relationship between t_{max} and c,

t_{max}=180.93+5.1842 c, R= –0.9901 (5~50 μg mL^{-1})

Discussion

The experiment indicated that low concentration of zinc(II) has stimulating action on *E. coli* growth, and high concentration of zinc(II) can inhibit *E. coli*. The percent of inhibition of *E. coli* increased with the increasing of zinc(II) concentration.

At low concentration, zinc(II) is made use of the cells and protects their integrated property. So it can make the cells have appropriate flow quality and is beneficial to the synthesis of DNA and RNA in the cells, so that it is important to preserve the regular growth and the biological activity of the cells. While at the high concentration, zinc(II) has inhibitory action on growth. It might be explained that zinc(II) acts on the mercapto of the biological macromolecule and make the proteins wrecked and the proliferation is rejected. The growth rate of the cells decreases. Factors that determine the characteristics of a dose-response curve are the toxicant's mode of action in cells, its number of target sites, and its affinity for those target sites. Zinc(II) may be combined with sulfhydryl groups on membrane proteins in all the cellular levels, resulting in cross-linking and inactivation. This changes cell membrane permeability and disrupts transport of nutrients and waste across the membrane. The toxicity of a toxicant for cells depends on its oxidation state, speciation, and the stability and solubility of its compounds. Some studied results showed a correlation between toxicity and sulfhydryl affinity and it is suggested that the cross-linking of membrane proteins is a major factor in the toxic effects of materials [12, 13].

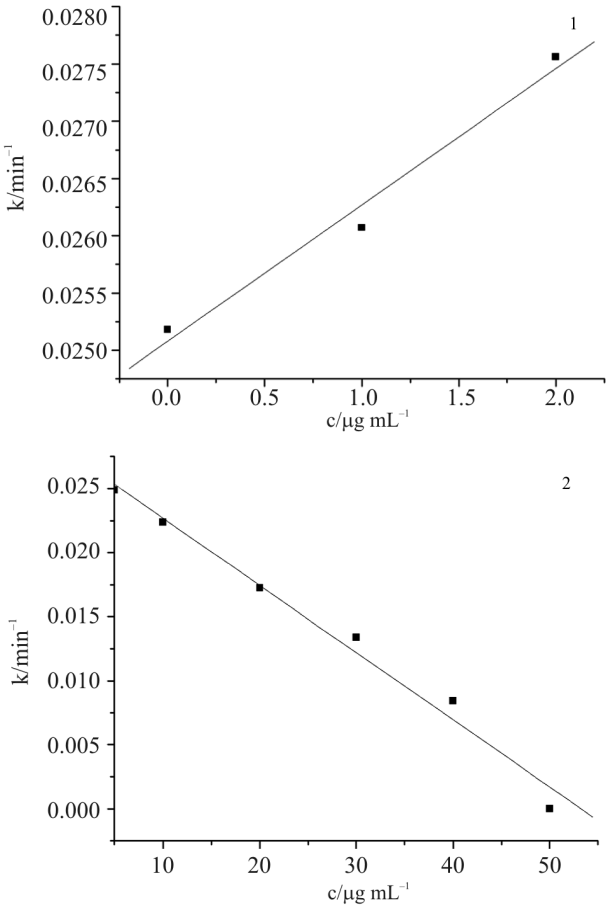

Fig. 2 Relationship between the growth rate constant (k) and c
1 – (k~c (0– ~2 μg mL^{-1}); 2 – k~c (5~50 μg mL^{-1})

Microcalorimetry can provide both thermodynamic and kinetic information. Calorimetry can enhance the accuracy of the determination of the physi-

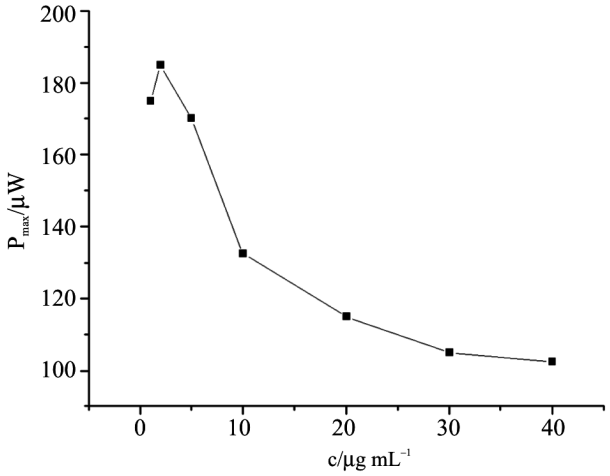

Fig. 3 Relationship between the maximum power in the log phase (P_{max}) and c (0–40 μg mL^{-1})

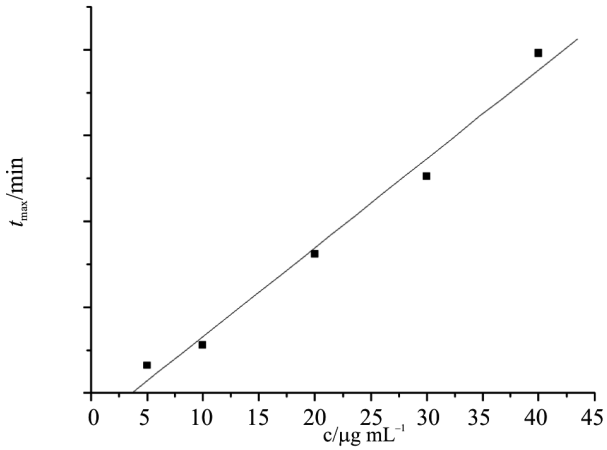

Fig. 5 Relationship between the times of peak of maximum power in the log phase (t_{max}) and c (5–40 μg mL^{-1})

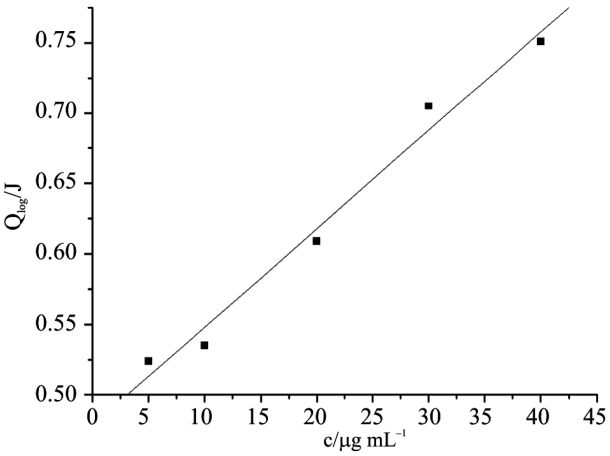

Fig. 4 Relationship between the heat output in the log phase Q_{log} and c (5–40 μg mL^{-1})

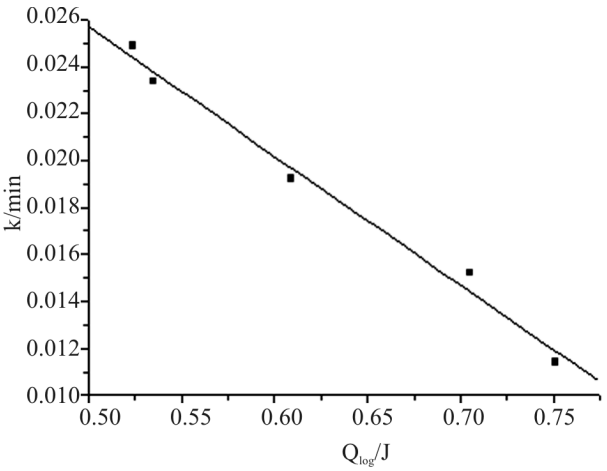

Fig. 6 Relationship between the growth rate constant (k) and the heat output and in the log phase (Q_{log})

Table 2 Experimental result of effects of zinc(II) on *E. coli* growth

c/μg mL^{-1}	k/min^{-1}	R	I/%	t_G/min	Q_{log}/J	P_{max}/μw	t_{max}/min
0	0.02518	0.9905	-	27.4	0.56	202.5	273
1	0.02607	0.9954	-3.5	26.5	0.48	180	198
2	0.02756	0.9933	-9.4	25.0	0.47	187.5	220
5	0.02489	0.9898	1.2	27.7	0.52	170	216
10	0.02237	0.9969	11.2	30.8	0.54	132.5	228
20	0.01925	0.9962	23.6	35.8	0.69	115	280
30	0.01641	0.9971	34.8	42.0	0.71	105	326
40	0.01143	0.9987	54.6	60.4	0.75	102.5	398
50	–	–	–	–	–	–	–

ological activity of the cultures. The calorimetric determination of toxicity results in lower as well as higher values of standard data within the range of variances of toxicity determinations in different laboratory by use of the same one standard method [14].

Calorimetry has been proved to be a useful tool for measuring the energy flow in natural samples. It is adaptable to toxicity studies in any type of cells. The advantage of calorimetry is that it measures the total thermal energy flow, and calorimetry in this respect is its non-specificity. By combining calorimetry with other specific methods, several different and important goals might be reached in studying the energy flow in natural environments [14].

Acknowledgements

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