J Therm Anal Calorim (2014) 115:2145–2149
DOI 10.1007/s10973-013-3324-8

Studies on the nongrowth metabolism of the different strains of *Tetrahymena* cells by isothermal microcalorimetry

X. J. Chen · W. S. Feng · Y. H. Yu

Received: 25 March 2013 / Accepted: 2 July 2013 / Published online: 30 July 2013

Abstract The nongrowth metabolic processes of *Tetrahymena* strains with the different cell densities were monitored by isothermal microcalorimetry, including *Tetrahymena thermophila* BF_1, *Tetrahymena thermophila* SB210, *Tetrahymena pyriformis* GL, the mixed cells of *T. thermophila* BF_1 and *T. thermophila* SB210, and the mixed cells of *T. thermophila* BF_1 and *T. pyriformis* GL. All the typical power–time curves showed a decreasing trend on the whole. It was found that total heat production (Q_t) and maximum heat output (P_m) decreased significantly with the decrease of cell density. Cell density did not influence significantly the maximum heat output per cell (P_{cell}) of *Tetrahymena* cells, which was probably due to enough oxygen. The P_{cell} and metabolic decrease rate constant (K) values for the mixture of *T. thermophila* BF_1 and *T. pyriformis* GL, and that of *T. thermophila* BF_1 and *T. thermophila* SB210 were similar to the mean values of P_{cell} and K for the corresponding single *Tetrahymena* strain, respectively. It was speculated that the cell mixing did not possibly influence significantly their P_{cell} and K values because of no competition for nutrition, oxygen, and room, and the low conjunction percentages.

X. J. Chen (✉)
Key Laboratory of Ecological Impacts of Hydraulic-Projects and Restoration of Aquatic Ecosystem of Ministry of Water Resources, Institute of Hydroecology, Ministry of Water Resources and Chinese Academy of Sciences, Wuhan 430079, People's Republic of China
e-mail: ihe2012@mail.ihe.ac.cn

W. S. Feng · Y. H. Yu (✉)
Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, People's Republic of China
e-mail: yhyu@ihb.ac.cn

Keywords Isothermal microcalorimetry · *Tetrahymena* · Nongrowth metabolism · Power–time curves · Power outputs

Introduction

All living systems can produce heat in the metabolic processes, which can be directly monitored by calorimetry [1]. With the development of the microcalorimetry, it has been used widely to monitor the growth metabolism of many living organisms and applied in many fields, such as physiology, pharmacology, ecology, and environmental sciences [2–7].

Besides the growth metabolism, the living organisms have the nongrowth metabolism. Without the supply of nutrients, cells can carry out the nongrowth metabolic activities with the low level based on their own intracellular storage of nutrients. The studies on the heat produced by the nongrowth metabolic processes will be useful for our understanding of the responses of cell metabolism to some adverse environments. Although microcalorimetry has a wide application in monitoring the growth metabolism of cells, it has scarcely been used to study the nongrowth metabolic processes of cells, except *Escherichia coli* [8], *Chlorella vulgaris* [9], and *Amoeba proteus* cells [10].

With many good features, *Tetrahymena* have been the common unicellular model organisms in many fields including cell biology, genetics, and environmental sciences [11]. Besides, the growth metabolism of *Tetrahymena* has been studied by microcalorimetry [5, 6]. The present study aims to record the heat production during the nongrowth metabolic processes of the different strains of *Tetrahymena* cells. The effects of the cell density and mixing of the different *Tetrahymena* strains on the

nongrowth metabolism of *Tetrahymena* cells have also been investigated.

Experimental

Species and culture medium

Tetrahymena thermophila BF_1 were provided by East China Normal University. *Tetrahymena pyriformis* GL was provided by PhD. Tie Yang was from the Institute of Zoology, Chinese Academy of Sciences. *Tetrahymena thermophila* SB210 were provided by Prof. Eduardo Orias from the University of California Santa Barbara, USA. The cells were cultured at 28 °C in a liquid medium containing 2 % (w/v) proteose peptone (Oxoid), 0.1 % yeast extract (Oxoid), and 0.5 mM $FeCl_3$. After culturing the *Tetrahymena* cells at 28 °C for about 3 days in the tubes, the cells attained the stationary stage and were prepared for microcalorimetric measurements.

Calorimeter

The calorimeter is an eight-channel TAM Air isothermal heat conduction calorimeter 3114/3236 (Thermometric AB, Sweden). The microcalorimetric channels are in a single removable block contained in an air thermostat that keeps the temperature within ±0.02 °C. Each channel consists of a sample and a reference vessel. The limit of detection is 2 μW, and the baseline deviation over 24 h is ±5 μW.

Microcalorimetric measurement of the single strain with the different cell densities

The stationary-stage cells of three *Tetrahymena* strains were, respectively, collected by centrifugation (5 min/8,000 rpm) including *T. thermophila* BF_1, *T. pyriformis* GL, and *T. thermophila* SB210. After being centrifuged, the cells were cleaned three times by sterile water to remove all medium. Then, the suspended cells were starved for 13 h. Subsequently, 3–5 μL cell suspension was extracted and killed by Lugol's iodine (40 g iodine and 60 g potassium iodide in 1 L distilled water). The dead cells were counted with an optical microscope at 10× magnification. The counted cell number was used to calculate the cell density. Then, the starved cell suspension was diluted with sterile water in accordance with the gradient of 1, 0.75, 0.5 and 0.25 (e.g. for GL strain, the cell densities of the diluted suspensions were 6.4, 4.8, 3.2 and 1.6 × 10^4 mL^{-1}, respectively). Finally, the 5-mL diluted cell suspensions of each gradient were put into the 20 cm^3 glass ampoules for microcalorimetric measurements at 28 °C. The power–time curves of *Tetrahymena* cells at 28 °C were recorded every minute by means of the Picolog software supplied with TAM Air. The values of metabolic variables for cells were obtained from two independent curves.

Microcalorimetric measurement of the mixed strains with the different cell densities

The stationary-stage cells were collected, cleaned, and starved by the same procedure as above. Afterward, the starved cell suspensions of three strains were, respectively, sampled for cell counting. Then, *T. thermophila* BF_1 cells were mixed with *T. thermophila* SB210 *T. pyriformis* GL cells with the same cell number. The mixed cell suspension was diluted with sterile water in accordance with the gradients of 1, 0.75, 0.5, and 0.25. Finally, the 5 mL diluted mixed cell suspensions of each gradient were put into the 20 cm^3 glass ampoules for microcalorimetric measurements at 28 °C. The power–time curves of *Tetrahymena* cells at 28 °C were recorded every minute by means of the Picolog software supplied with TAM Air. The values of metabolic variables for the mixed cells were also obtained from two independent curves. After the end of microcalorimetric measurements, the conjugation of *Tetrahymena* cells was observed under a microscope.

Data analyses

The power–time curves and microcalorimetric variables were obtained by Origin 7.0. The data were given as the arithmetic mean ± standard derivation. The one-way ANOVA statistical method was used to assess the significance of differences in the measured variables between the samples at $P \leq 0.05$.

Results and discussions

Nongrowth metabolic power–time curves of *Tetrahymena* strains

The typical power–time curves for the nongrowth metabolism of three *Tetrahymena* strains and the mixed strains at 28 °C can be observed from Figs. 1, 2, 3, 4, and 5. These curves described the different evolution of the thermal effects in the nongrowth metabolic processes of *Tetrahymena* strains. The nongrowth metabolism of *T. pyriformis* GL presented a typical decreasing trend. After a decreasing period for about 12 h, the power–time curve of *T. thermophila* BF_1 cells had a steady metabolic period from the 12th to 13th h, and then their power outputs decreased gradually. A small peak in the power–time curves of *T. thermophila* BF_1 appeared possibly because of the fluctuation of instrument. The power output of *T. thermophila* SB210 cells was observed to be

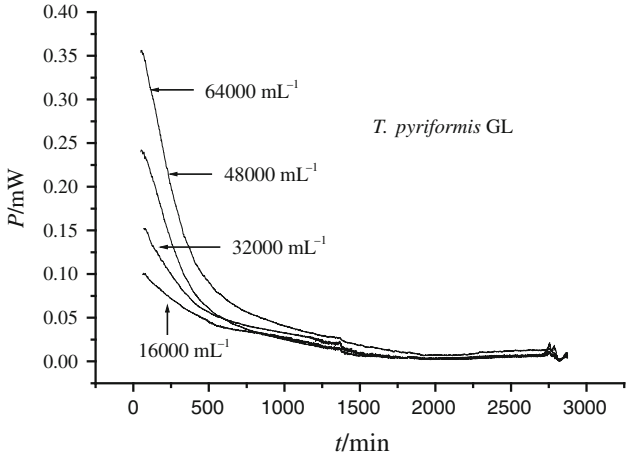

Fig. 1 Nongrowth metabolic power–time curves of *T. thermophila* GL with the different cell densities

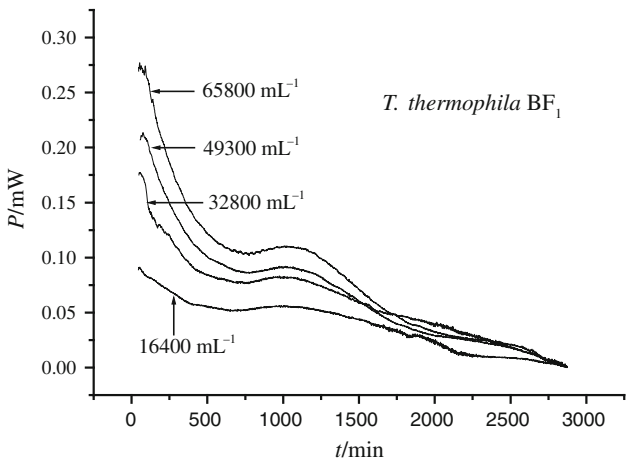

Fig. 2 Nongrowth metabolic power–time curves of *T. thermophila* BF_1 with the different cell densities

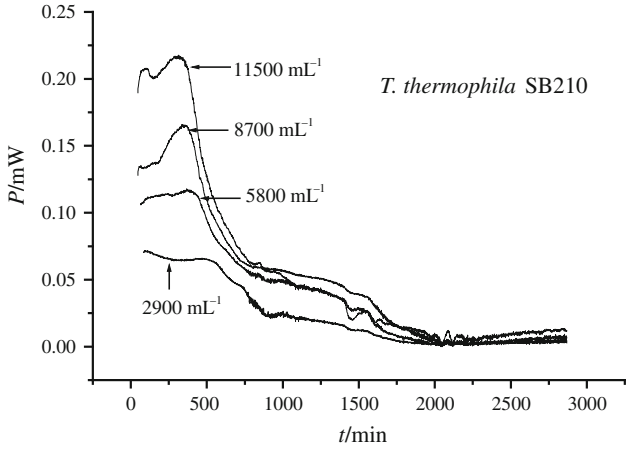

Fig. 3 Nongrowth metabolic power–time curves of *T. thermophila* SB210 with the different cell densities

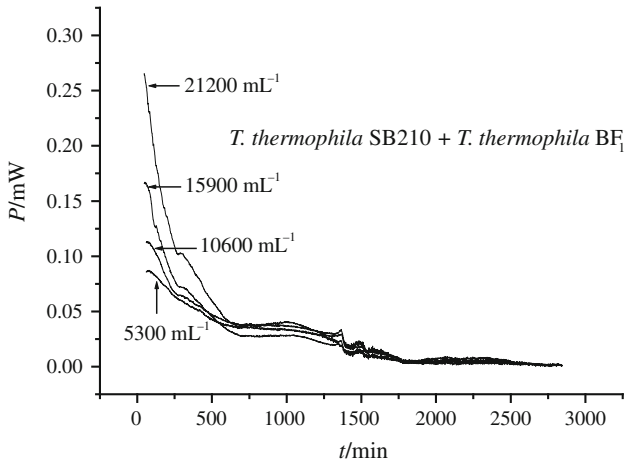

Fig. 4 Nongrowth metabolic power–time curves for the mixed cells of *T. thermophila* BF_1 and *T. thermophila* SB210 with the different cell densities

steady during the beginning period for 9 h, and then decreased gradually. The power–time curves for the mixture of *T. thermophila* BF_1 and *T. thermophila* SB210 cells had a decreasing metabolic stage before the 11th h, a steady metabolic stage from 11th to 16th h, and then a final decreasing metabolic stage. However, for the mixture of *T. thermophila* BF_1 and *T. pyriformis* GL cells, their power outputs declined continuously in the whole process.

From the power–time curves of *Tetrahymena* strains, it was found that there were differences in their nongrowth metabolic processes. However, the differences of cell densities had no significant effects on the shape of the power–time curves. On the whole, the power outputs had gone through a gradual downward process and declined to be the baseline level finally. Apparently, the decrease of the cells' own energy substances led to a gradual reduction in the metabolic level. Finally, the depletion of their own nutrients made their metabolism extremely weak.

Nongrowth metabolic properties of *Tetrahymena* strains

From these typical curves, the nongrowth metabolic properties of *Tetrahymena* strains were obtained and are presented in Table 1, including maximum power output (P_m), maximum power output per cell (P_{cell}), the decline rate constant of nongrowth metabolism during the decreasing period (K), and total heat production (Q_t).

From the values of the metabolic properties, it was found that P_m and Q_t decreased significantly with the decrease of cell densities. Except for the groups with about 16,000 mL^{-1} cell densities, the K values of the other groups for *T. pyriformis* GL and *T. thermophila* BF_1 did not decrease significantly, compared with the largest cell

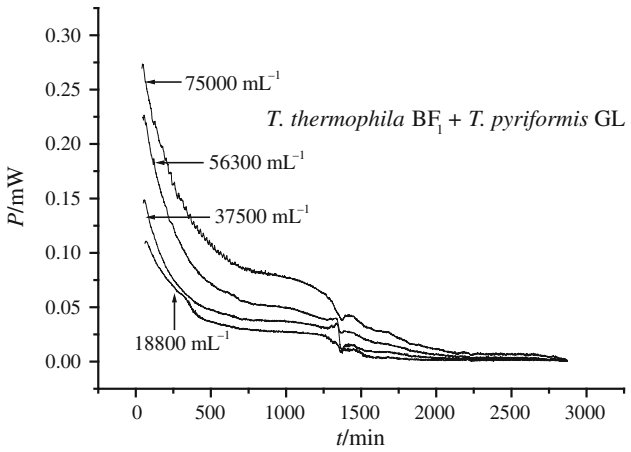

Fig. 5 Nongrowth metabolic power–time curves for the mixed cells of *T. thermophila* BF_1 and *T. thermophila* GL with the different cell densities

density groups. For SB210, BF_1 + SB210 and BF_1 + GL, there were no significant differences in K values between the largest cell density groups and the other groups.

In the present study, it was worth noting that *Tetrahymena* cells in P_{cell} do not change significantly with the different cell densities. Xie et al. [8] showed that the nongrowth metabolism power output per cell for *E. coli* with 2.9, 1.5, 0.72, and 0.37 × 10^8 cell numbers were all 0.045 pw. However, the previous microcalorimetric studies about the nongrowth metabolism of *Chlorella* [9] and *Amoeba* [10] cells revealed that the heat production per cell increased with the decreasing cell density. The existence of mutual inhibition between cells was speculated, and the effect was called crowding effect [9, 10, 12]. In the absence of nutrients, the limiting factor for the cell metabolism was oxygen or room. Pace and Lyman [13] had reported that the average oxygen consumption of the hungry *Tetrahymena geleii* cells in the different growth periods was all <400 μL h^{-1} per million cells. In the present study, the peak time for the maximum power output of *Tetrahymena* cells was <15 h. Making reference to 400 μL h^{-1} oxygen consumption per million cells, *Tetrahymena* cells oxygen consumption within 15 h was <2.25 mL, which was lower than the oxygen volume (about 3 mL) in the experimental ampoule bottles. Therefore, no significant changes of P_{cell} with the cell density were observed maybe because of the sufficient oxygen.

The P_{cell} values of *T. thermophila* SB210, *T. thermophila* BF_1, and *T. pyriformis* GL cells were about 4.26, 1.07, and 1.09 nW, respectively. The power output per cell of *T. pyriformis* ST was 3.3 nW measured at 25 °C [14]. Liu

Table 1 Nongrowth metabolic characteristics of different *Tetrahymena* strains

Strain	D/10^4 mL^{-1}	P_m/mW	P_{cell}/nW	K/10^{-3} min^{-1}	Q_t/J
GL	6.40	0.36 ± 0.09	1.12 ± 0.29	−3.33 ± 0. 27	9.00 ± 2.14
GL	4.80	0.24 ± 0.03	1.01 ± 0.13	−3.41 ± 0.11	5.78 ± 0.49*
GL	3.20	0.15 ± 0.02*	0.96 ± 0.12	−2.42 ± 0. 53	4.96 ± 0.19*
GL	1.60	0.10 ± 0.02*	1.27 ± 0.24	−1.81 ± 0.51*	3.84 ± 0.59*
BF_1	6.58	0.35 ± 0.10	1.05 ± 0.30	−2.28 ± 0.50	14.03 ± 0.49
BF_1	4.93	0.23 ± 0.02	0.93 ± 0.09	−1.90 ± 0.18	11.86 ± 0.88*
BF_1	3.28	0.18 ± 0.00*	1.07 ± 0.01	−1.68 ± 0.04	10.35 ± 0.00*
BF_1	1.64	0.10 ± 0.01*	1.21 ± 0.14	−1.30 ± 0.02*	6.22 ± 0.45*
SB210	1.15	0.21 ± 0.01	3.68 ± 0.13	−2.74 ± 0.18	9.55 ± 0.24
SB210	0.87	0.17 ± 0.00*	3.88 ± 0.08	−2.75 ± 0.03	8.47 ± 0.23
SB210	0.58	0.12 ± 0.00*	3.99 ± 0.14	−2.14 ± 0.16	6.09 ± 0.69*
SB210	0.29	0.08 ± 0.02*	5.49 ± 0.16	−3.01 ± 0.04	3.81 ± 0.31*
BF_1 + SB210	2.12	0.27 ± 0.11	2.51 ± 1.00	−2.93 ± 0.68	6.04 ± 0.95
BF_1 + SB210	1.59	0.17 ± 0.03	2.10 ± 0.36	−2.71 ± 0.44	4.35 ± 0.04*
BF_1 + SB210	1.06	0.12 ± 0.02*	2.19 ± 0.39	−1.84 ± 0.57	4.45 ± 0.13*
BF_1 + SB210	0.53	0.09 ± 0.02*	3.31 ± 0.84	−1.62 ± 0.55	3.74 ± 0.02*
BF_1 + GL	7.50	0.26 ± 0.01	0.70 ± 0.04	−2.07 ± 0.06	9.70 ± 0.45
BF_1 + GL	5.63	0.24 ± 0.01	0.84 ± 0.05	2.52 ± 0.18	7.41 ± 0.73*
BF_1 + GL	3.75	0.16 ± 0.01*	0.83 ± 0.05	2.24 ± 0.51	5.42 ± 1.26*
BF_1 + GL	1.88	0.10 ± 0.02*	1.04 ± 0.19*	1.98 ± 0.97	3.83 ± 0.57*

BF_1 + SB210 means the mixed cells of *T. thermophila* BF_1 and *T. thermophila* SB210. BF_1 + GL means the mixed cells of *T. thermophila* BF_1 and *T. pyriformis* GL. D is cell density. P_m is maximum power output. P_{cell} is maximum power output per cell. K is metabolic decrease rate constant during the decreasing period, Q_t is total heat production. The data marked with * are significant compared with the groups with the largest densities

and Xie had both monitored the growth metabolism of *T. pyriformis* GL in the different culture medium at 25 °C, and the power outputs per cell obtained by them were 2.32 and 3.18 nW, respectively [15, 16]. Compared with the previous studies, the power outputs per cell in the growth metabolism of *T. pyriformis* at lower temperature (25 °C) were significantly higher than P_{cell} value (1.09 nW) obtained at 28 °C in the present study. The growth metabolic levels with the enough nutrient supply was obviously higher than the nongrowth metabolic levels with no nutrient supply. The power output per cell for *A. proteus* at 25 °C was about 0.9 nW after 3, 4, and 5 days of starvation [10], which was similar to those for *T. thermophila* BF_1 and *T. pyriformis* GL. However, for *Amoeba* cells starved for 7 and 10 days, the power values per cell were, respectively, 0.54 and 0.46 nW, significantly lower than the values for cells starved for 3, 4, and 5 days [10]. The mean power outputs per cell of the nongrowth metabolism for *E. coli* at 37 °C and *C. vulgaris* at 25 °C were 0.045 and 9.22 pW, respectively [8, 9], significantly lower than the corresponding values for *Tetrahymena* and *Amoeba* cells.

The P_{cell} and K values for the mixture of *T. thermophila* BF_1 and *T. pyriformis* GL, and *T. thermophila* BF_1 and *T. thermophila* SB210 were similar to the mean values of P_{cell} and K for the corresponding single *Tetrahymena* strain, respectively. It was speculated that the mixing of *Tetrahymena* strains possibly did not influence significantly the P_{cell} and K of each strain. No competition for nutrition, oxygen, and room might have led to these results. By the microscope observation, the conjugation was found between *T. thermophila* SB210 and *T. thermophila* BF_1 after the microcalorimetric measurements. Interestingly, the conjugation also did not affect their metabolism significantly, which was possibly because the conjugation percentage was very low in the microcalorimetric processes. A further study is still needed to clarify what effects the mixing of the different *Tetrahymena* strains have on their single strain metabolism if the conjunction percentage is high.

Conclusions

In the present study, the typical power–time curves of the nongrowth metabolic processes of three *Tetrahymena* strains and their mixed *Tetrahymena* strains were obtained by isothermal calorimetry, all of which showed a decreasing trend on the whole. It was found that Q_t and P_m decreased significantly with the decreasing cell density. However, cell density did not affect significantly P_{cell}, which was different from the previous results for *A. proteus* and *C. vulgaris*. The P_{cell} values of *T. thermophila* SB210, *T. thermophila* BF_1, and *T. pyriformis* GL cells were 4.26, 1.07, and 1.09 nW, respectively. Furthermore, mixing *T. thermophila* BF_1 with *T. thermophila* SB210, and *T. pyriformis* GL, respectively, did not possibly influence significantly the P_{cell} and K values of each strain.

Acknowledgements The authors gratefully acknowledge the financial support of the Nature and Science Foundation of China (51009099, 30170136). The authors thank Dr. Peng Liu and Prof. Xi Li in Wuhan University of Technology for their providing the calorimeter. We would also like to express our gratitude to Mr Yutian Gong for his helps in improving the English of the paper.

References

1. Wadsö I. Isothermal micromicrocalorimetry in applied biology. Thermochim Acta. 1995;394:305–11.
2. Bjurman J, Wadsö L. Microcalorimetric measurements of metabolic activity of six decay fungi on spruce wood as a function of temperature. Mycologia. 2000;9291:23–8.
3. Kemp RB, Lamprecht I. La vie est donc un feu pour la calorimetrie: half a century of. microcalorimetry—Ingemar Wadsö at 70. Thermochim Acta. 2000;348:1–17.
4. Chen XJ, Feng WS, Shen YF, Liu Y, Miao W, Yu YH. Application of polyurethane foam units and microcalorimetry to microbial monitoring in Lake Donghu. Thermochim Acta. 2005;438:63–9.
5. Chen XJ, Feng WS, Miao W, Yu YH, Shen YF, Wan CY, Peng JH. A microcalorimetric assay of *Tetrahymena thermophila* for assessing tributyltin acute toxicity. J Therm Anal Calorim. 2008;94:779–84.
6. Fang TT, Li X, Wang CG, Zhang ZJ, Zhang T, Zeng JM, Liu P, Zhang CC. The action of norfloxacin complexes on *Tetrahymena* investigated by microcalorimetry. J Therm Anal Calorim. 2012; 109(1):433–9.
7. Wang CY, Xu F, Sun LX, Sun YJ, Qiu SJ, Zhao ZB, Tan HD, Wang S. Influences of levofloxacin salts on the metabolism of *Escherichia coli* by microcalorimetry. J Therm Anal Calorim. 2013;111(1):959–63.
8. Xie CL, Tang HK, Sun DY, Qu SS, Liu HS, Liao YT. Thermochemical studies of different types of metabolism of cell I. measurement of the heat output of bacterial growth, non-growth and endogenous metabolism. Acta Phys-Chim Sin. 1996;12(3):280–3.
9. Liu Y, Tzitschung TT, Cheng Z, Qu SS, Shen P. Microcalorimetric studies on the metabolism of *Chlorella vulgaris*. Chemosphere. 2000;40(8):845–9.
10. Nässberger L, Monti M. Assessment of overall metabolism in *Ameboa Proteus* measured by a microcalorimetric method. Protoplasma. 1984;123:135–9.
11. Shen YF. Protozoology. Beijing: Science press; 1999.
12. Yan CN, Liu Y, Song ZH, Qu SS. Microcalorimetric studies of different types of metabolism of cells II. non-growth aerobic metabolic characteristics of resting cells. Phys-Chim Sin. 1997;13(5):477–80.
13. Pace DM, Lyman ED. Oxygen consumption and carbon bioxide elimination in *Tetrahymena geleii* furgason. Biol Bull. 1947;92: 210–6.
14. Lloyd DC, Phillips A, Statham M. Oscillations of respiration, adenine nucleotide levels and heat evolution in synchronous cultures of *Tetrahymena pyriformis* ST prepared by continuous-flow selection. J Genet Microbiol. 1978;106:19–26.
15. Liu Y, Sun DY, Yu YH, Qu SS, Shen YF. Microcalorimetric investigations of thermochemical characteristics of the growth metabolism of *Tetrahymena pyriformis*. Chem J Internet. 2002;4(1):1–6.
16. Xie CL, Sun DU, Song ZH, Qu SS, Guo Y, Li B. Microcalorimetric studies on *Tetrahymena pyriformis*: part 1. Growth metabolic power and thermal equations. Thermochim Acta. 1991; 183:117–23.