Journal of Thermal Analysis, Vol. 30 (1985) 1397—1402

MICROCALORIMETRIC AND THERMODYNAMIC STUDIES OF THE EFFECT OF TEMPERATURE ON THE ANAEROBIC GROWTH OF SERRATIA MARCESCENS IN A MINIMAL GLUCOSE-LIMITED MEDIUM

*J. Bermudez** and *J. Wagensberg***

*DPT. FISICA MEDICA, FACULTAT DE MEDICINA, UNIVERSITAT DE BARCELONA, CASANOVA 143, 08036 BARCELONA
**DPT. TERMOLOGIA, FACULTAT DE FISICA, UNIVERSITAT DE BARCELONA, DIAGONAL 647, 08028 BARCELONA, SPAIN

Calorimetric and growth data are reported for the anaerobic growth of a strain of Serratia marcescens in a glucose-limited medium over the temperature range from 20 to 32°. Power-time curves and growth curves evolve in a well-known regular way *vs.* increasing environmental temperature. The growth rate constants in the heat dissipation sense $k_Q = \dot{Q}/Q$ or, alternatively, in the biomass production sense $k_m = \dot{m}/m$ are maximum at 30°, constant k_m being greater than constant k_Q throughout the studied temperature interval. On the other hand, the metabolic efficiency is defined in a thermodynamic context and found to be maximum at 28°.

The growth temperature has a crucial influence on both the growth characteristics and the metabolism of microorganisms [1–3]. In particular, it clearly plays a relevant role as concerns the competition of different species of bacteria growing in the same environment.

This investigation was undertaken with cells of Serratia marcescens in a minimal glucose-limited ambient at different temperatures, in order to explore the optimization of two efficiencies, one relating to the simple kinetics of biomass production and the other to the degree of thermodynamic reversibility exhibited by the ensemble of metabolic processes.

Material and methods

Organism and culture medium. The organism is a strain of Serratia marcescens (ATCC 274), a facultative bacterium able to denitrify. Stock cultures were maintained on nutrient agar slants at 4° at transferred monthly.

Experimental growth was carried out in a minimal salt/glucose medium with the following composition g/l of double distilled water): D-glucose 2, NaCl 5, $Na_2HPO_4 . 12H_2O$ 11, KH_2PO_4 2.7, $(NH_4)_2SO_4$ 1, $CaCl_2$ 0.01, $MgSO_4 . 7H_2O$ 0.1,

John Wiley & Sons, Limited, Chichester
Akadémiai Kiadó, Budapest

$FeSO_4.7H_2O$ 25×10^{-5}. This medium is limiting only in carbon source. The pH was adjusted at 6.8 and remained constant until the last part of the growth and in any case within the interval 6.5–6.8 (Fig. 1b).

Experimental system. The microcalorimeter used was a heat flux calorimeter of Calvet type with a sensitivity of 60.6 mV/W.

Biomass production was determined by means of the optical density at 560 nm with a Perkin–Elmer 3000 spectrofluorimeter The optical density was calibrated *vs.* the cell-protein contents of the culture [4].

Glucose concentration was determined according to the GOD-period method (Boehringer, Mannheim), and the oxygen concentration using an Orbisphere 6203 polarographic indicator.

Experimental conditions. The prewarmed medium was inoculated with a diluted suspension of mid-log cells of a second preculture. The initial density of the inocula was about 10^4 cell/ml. The calorimeter and fluorimeter vessels were completely filled (6.6 ml) with an inoculated and homogenized medium and then hermetically closed. By this means, essentially anaerobic growth conditions were achieved [3].

The experimental device is therefore prepared for the simultaneous recording of the changes in time of four parameters: the heat output, the biomass production and the glucose and oxygen consumptions. Details of this device were reported previously [5]. Figure 1 shows the results of a typical experiment during the growth of Serratia marcescens ATCC 274 at 28°. At least three such measurements were made at each temperature in order to determine the values of the growth and metabolic efficiencies (Table 1). Note that the oxygen curves clearly locate the end of the early aerobic phase.

Table 1 Variation of growth parameters with temperature for the anaerobic growth of Serratia marcescens ATCC 274 in a minimal-glucose medium

Temperature T,	Heat dissipation rate k_Q, $10^{-5}\ s^{-1}$	Biomass production rate k_m $10^{-5}\ s^{-1}$	Yield coefficient Y_G, g/mol	Enthalpic coefficient K, kJ/mol
20	3.32±0.86	4.57±0.36	6.06±0.92	93.5± 2.2
22	3.97±0.03	5.51±0.22	6.60±0.14	86.0±11.0
24	3.86±0.14	6.11±0.19	6.30±0.09	
26	5.10±0.28	8.09±0.12	7.55±0.07	70.5± 1.7
28	6.18±0.34	8.93±0.21	10.25±0.64	70.9± 1.7
30	8.74±1.27	11.41±0.40	10.03±0.42	82.4± 1.6
32	7.62±0.08	11.27±0.27	8.00±0.10	90.8± 2.2

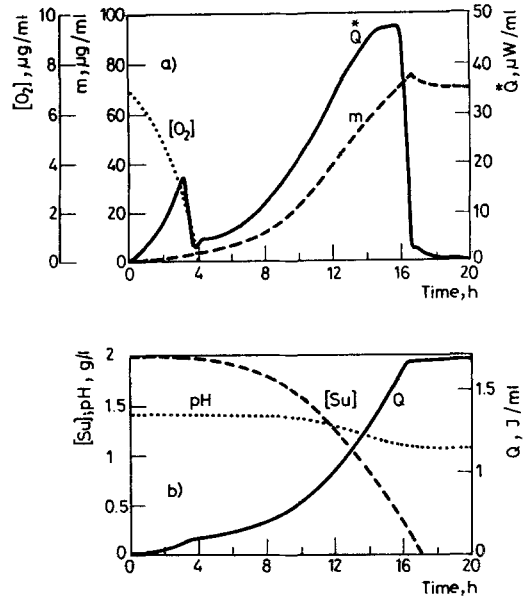

Fig. 1 Growth parameters corresponding to Serratia marcescens ATCC 274 growing in a minimal-glucose medium at 28°C

Results and discussion

The profiles of the recorded power-time ($p-t$) curves depended strongly on the growth temperatures. Figure 2a shows the consistent heat response within the limits 20 and 32°. At all temperatures, the power increased exponentially during the logarithmic anaerobic growth phase and always attained a maximum value at the end of this phase, thereafter decreasing to the baseline. In particular, we focus our attention on this exponential behaviour, that can be located at each temperature. For instance, Fig. 1 provides the necessary information to locate the exponential anaerobic growth (here centred at 10 h) at a temperature of 28°. The aim of our approach is to assume that this situation represents a stage that could be maintained indefinitely under open conditions; in other words, we consider this phase as a virtual thermodynamic stationary state. We can therefore determine experimentally the following parameters associated with this previously located state at each temperature: the constant kinetic and heat rates, $k_m = \dot{m}/m$ and $k_Q = \dot{Q}/Q$, and the constant growth yield and enthalpic coefficient [6, 7], defined respectively as $Y_G = \mathrm{d}m/\mathrm{d}[Su]$ and $K = \mathrm{d}Q/\mathrm{d}[Su]$, Q being the dissipated heat, m the biomass and (Su) the glucose concentration (Table 1).

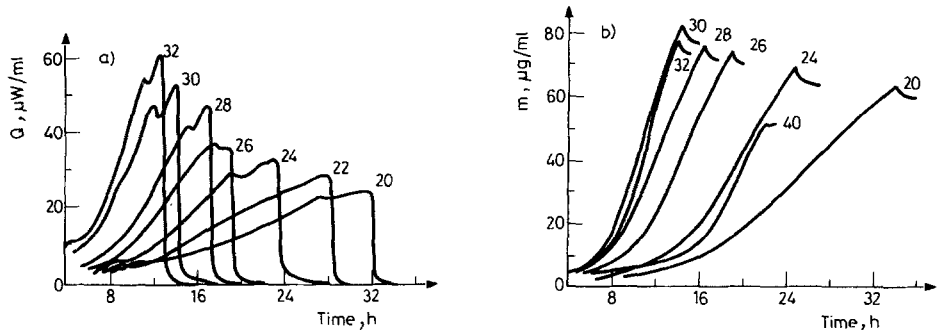

Fig. 2 The effect of growth temperature on (a) power-time curves and (b) biomass production of Serratia marcescens ATCC 274 growing anaerobically in a minimal-glucose medium

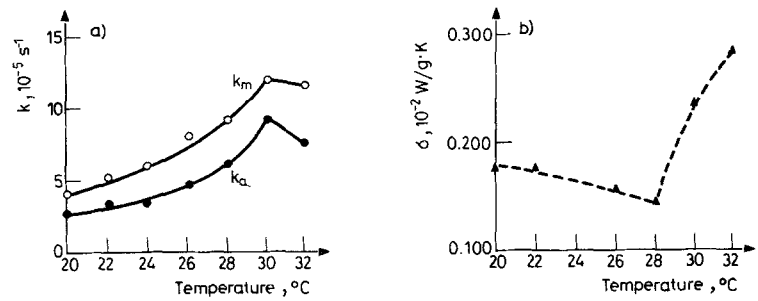

Fig. 3 (a) The biomass production rate k_m, ○, and the heat dissipation rate k_Q, ●, *vs.* temperature and (b) the entropy production per unit of produced biomass σ, ▲, *vs.* temperature of Serratia marcescens ATCC 274 growing in a minimal-glucose medium

We characterize this stationary state, at a given temperature T, both biologically and thermodynamically. We choose the biomass production rate k_m (or alternatively k_Q) as a relevant biological efficiency and the entropy production per unit of produced biomass, σ, as a significant metabolic thermodynamic efficiency. The specific entropy production can be measured—in the neighbourhood of the stationary state—by its metabolism as recorded by calorimetry [8]:

$$\sigma_{stat} = -\frac{1}{T}\left\{\frac{\dot{Q}}{m}\right\}_{stat} \tag{1}$$

where T is the externally imposed temperature and $(\dot{Q}/m)_{stat}$ the heat dissipation rate per unit of produced biomass for the observed stationary state. The stationary value of the specific entropy production can therefore be determined experimentally as a function of the stationary rates given earlier, i.e.:

$$\sigma_{stat} = \frac{1}{T} k_Q \frac{K}{Y_G} \tag{2}$$

This value represents the degree of irreversibility of the metabolic processes going on the system. On the other hand, the thermodynamic idea of adaptation, understood as the situation of minimum entropy production which is compatible with the imposed environmental constraints, provides a particular insight with regard to the biological adaptation [9].

Figure 3 shows the results of our investigation as concerns temperature. Figure 3a exhibits a maximum value for the kinetic rates k_m or k_Q at 30°. This is the optimum growth temperature, at which the metabolism of Serratia marcescens goes fastest. In a competitive situation, it represents an eventual advantage for a rapid colonization of the medium. Figure 3b exhibits the best thermodynamic conditions at 28°. This is the optimum growth temperature, at which the metabolism of Serratia marcescens has the most economic energetic costs. It represents a better coupling between anabolism and catabolism, that is to say, a higher biomass production per unit of consumed substrate (Y_G) and, therefore, another kind of advantage for the growing microorganism. Thus, microcalorimetry is, in particular, an accurate and direct tool for determination of the best thermodynamic growing conditions and this is of some relevance as concerns the microbiological strategy of adaptation.

References

1 R. H. Mennett and T. O. M. Nakayama, Appl. Microbiol., 22 (1971) 772.

2 S. C. Nichols and A. M. James, Microbios., 31 (1981) 153.

3 H. E. Reiling and H. Zuber, Arch. Microbiol., 136 (1983) 243.

4 O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, Biol. Chem., 193 (1951) 265.

5 J. Bermudez, J. G. Lorén and J. Wagensberg, Inv. Pesq., 48 (1984) 3.

6 B. Schaarschmidt and I. Lamprecht, Thermochim. Acta, 22 (1978) 333.

7 J. P. Belaich, J. C. Senez and M. Murgier, J. Bacteriol., 95 (1968) 1750.

8 I. Prigogine, Introduction to Thermodynamics of Irreversible Processes, Interscience, New York, 1962.

9 D. Lurié and J. Wagensberg, J. Theor. Biol., 78 (1979) 241.

Zusammenfassung — Kalorimetrische Werte und Wachstumsdaten werden für das anärobe Wachstum des Kulturstammes Serratia marcescens in einem Glukose-limitierten Medium im Temperaturbereich von 20—32° C angegeben. Leistungs-Zeit-Kurven und Wachstumskurven zeigen mit ansteigender Umgebungstemperatur den gut bekannten regulären Verlauf. Die Wachstumsgeschwindigkeitskonstanten (im Sinne der Wärmeableitung $k_Q = \dot{Q}/Q$ einerseits oder im Sinne der Produktion von Biomasse $k_m = \dot{m}/m$ andererseits) gehen bei 30 °C durch ein Maximum, wobei im untersuchten Temperaturintervall die Konstante k_m größer als K_Q ist. Der metabolitische Wirkungsgrad ist thermodynamisch definiert und weist ein Maximum bei 28 °C auf.

Резюме — Приведены калориметрические данные и данные анаэробного роста штаммов Serratia marcescens в интервале температур 20–32° и в среде с минимальной концентрацией глюкозы. Кривые энергия — время и кривые роста в зависимости от температуры были определены обычными методами. Константы скорости роста (в смысле рассеяния тепла $\kappa_Q = \dot{Q}/Q$ или же смысле получения биомассы $\kappa_m = \dot{m}/m$) были максимальными при 30°. Во всем изученном температурном интервале константа κ_m была больше, чем κ_Q. Но с другой стороны, метаболический выход, определенный в термодинамической связи, достигал максимума при 28°.