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Microcalorimetric study of the effect of temperature on microbial activity in soils

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Abstract

The effect of temperature on the microbial degradation of glucose in soil was studied in a conduction-type microcalorimeter. The microbial growth constant, μ, was calculated from the heat evolution associated with the degradation of carbon source, and the power–time curves recorded at different temperatures.

The activation energy was obtained from an Arrhenius-type equation and was found to be 39.57 kJ mol^{-1}. Based on the fact that the pattern of bacterial growth is similar to the transition-state theory for chemical process, values of activation entropy, $\Delta S^{\neq}$, and activation free energy, $\Delta G^{\neq}$, were calculated. These data can be very useful when studying bacterial growth thermodynamic properties in soil. © 1997 Elsevier Science B.V.

Keywords: Microbial growth; Microcalorimetry; Soils

1. Introduction

In two previous papers [1,2], we have used microcalorimetry to study microbial activity in soil. This method has proved to be a technique well-suited to measurements of microbial activity in complex systems, as only the change from the initial to the final state of the system contributes to the total heat effect [3,4].

The temperature at which microbial cells grow plays an important role on the physiological conditions of the microorganisms, resulting in the alteration of the growth rate and the degradation activity of organic substances [5,6]. The heat output, associated with overall metabolic processes taking place in a culture, can be continuously detected without disturbing the system [7]. In this way, microbial activity can be recorded in situ. This paper reports on the application of the microcalorimetric method to soil microbial systems in order to quantitatively characterize the influence of temperature on microbial degradation of glucose in soil. The influence of temperature on any reaction or process is determined by the so-called activation energy. For this reason, the apparent activation energy and the change in Gibbs energy for the degradation process were derived from the variation of growth rate with temperature.

2. Experimental

Samples of humic cambisol soil were collected from 10 randomly chosen points from different sites situated in Monte Pedroso (Santiago de Compostela,

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Table 1
Soil characteristics

Water-holding capacity	30.07%
Water content	22.35%
% C	10.27%
% N	0.69%
pH	4.32

Spain). Some soil characteristics are given in Table 1. The soil samples were collected from a depth of 5–15 cm, after removal of the top surface layer.

Following a statistical method, a large number of samples of every site were collected and then all of them were mixed in an attempt to obtain one bulk sample representing the entire zone under study. In order to keep its moisture content, the soil was stored in polyethylene bags and sent to the laboratory, where it was sieved (mesh size $2 \times 2\ mm^2$) and the water content, organic matter, nitrogen content, carbon content, pH and water-holding capacity measured following routine methods.

The remaining bulk sample was moistened to water-holding capacity, considering this water content as the optimum to maintain microbial growth. After preparation, the soil bulk sample was stored in closed polyethylene bags at 4°C for at least three months to attain equilibrium before being used in the calorimetric experiments. Such a treatment ensures satisfactory reproducibility of the measurements [2,3,5].

Heat production by the soil was measured in a microcalorimeter 2277 thermal activity monitor (Thermometric AB, Sweden), a commercial version developed by Suurkuusk and Wadsö [8]. This microcalorimeter has been designed to operate in a wide range of chemical and biological reactions, allowing measurements of both endothermic and exothermic processes.

Thereafter, 1 g soil samples at water-holding capacity were introduced into $5\ cm^3$ stainless steel ampoules. These ampoules were hermetically closed by Teflon sealing discs to avoid evaporation inside the apparatus. The base line was determined in the usual way, introducing 1 g of soil in the test ampoule and 1 g of distilled water in the reference tube [1,3,5]. Before each experiment, soil samples were amended with an amount of glucose, equivalent to 5% of their carbon content, in $0.2\ cm^3$ distilled water. Experiments were carried out at various temperatures, namely 288.15, 293.15, 298.15 and 303.15 K.

Because exponential growth is considered sufficient for our calculations, power–time curves were recorded only up to the maximum value of thermal power. This same is pointed out by Battley [9], when studying the growth of *Saccharomyces cerevisiae*, stating that the duration of an experiment must be limited and the practical limit in time for a microbial growth experiment is the point at which the slope of the line representing the (logarithm of the increase in mass)/time relationship ceases to be positive and drops to zero. Values of microbial growth rate constant, μ, activation energy, E_a, Gibbs free energy changes, $\Delta G^{\neq}$, and the entropy change, $\Delta S^{\neq}$, were calculated from the power–time curves recorded.

3. Results and discussion

Power–time curves for the microbial growth log phase and the corresponding carbon-source degradation, at the different above-mentioned temperatures, are shown in Fig. 1. The considerable length of exponential growth at 288.15 K can be observed.

Fig. 2 shows the straight lines obtained when representing the logarithm of heat flow vs. time. The microbial growth rate constants were obtained from the slopes of the corresponding lines. Table 2 gives values of microbial growth rate constant, μ, at different temperatures. It is observed that in the selected

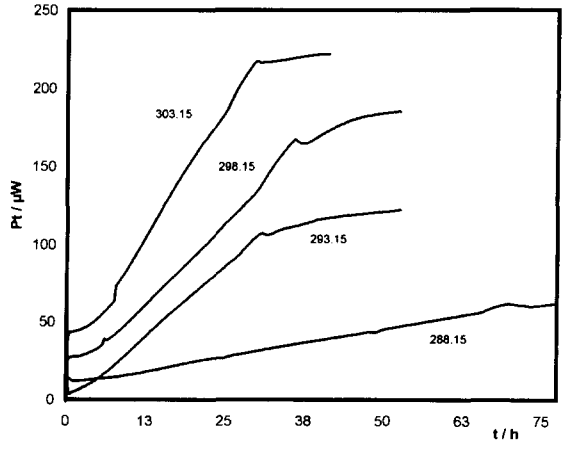

Fig. 1. *P–t* curves at various temperatures.

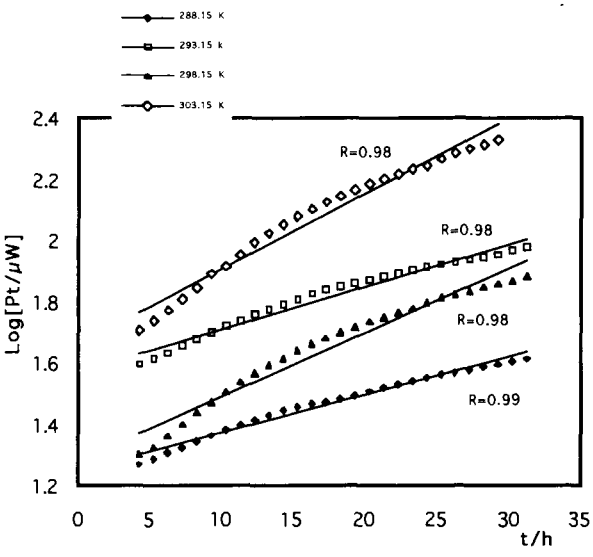

Fig. 2. Log P–t curves. Microbial growth rate constants can be obtained from the slopes of these curves.

Table 2
Values of microbial growth rate constant at various temperatures

T/K	μ/h^{-1}	r	Mean ± SD
	0.030	0.99	
288.15	0.021	0.99	0.026 ± 0.005
	0.028	0.98	
	0.031	0.99	
293.15	0.037	0.99	0.033 ± 0.005
	0.042	0.98	
	0.042	0.98	
298.15	0.049	0.98	0.047 ± 0.005
	0.051	0.99	
	0.057	0.99	
303.15	0.054	0.99	0.057 ± 0.004
	0.061	0.99	

temperature range, as expected, μ increases with temperature.

It is a well-known fact that the rate of chemical and physical processes increases with temperature. The influence of temperature on these process is determined by the activation energy. This activation energy is defined by the Arrhenius equation, which relates the reaction-rate change with temperature.

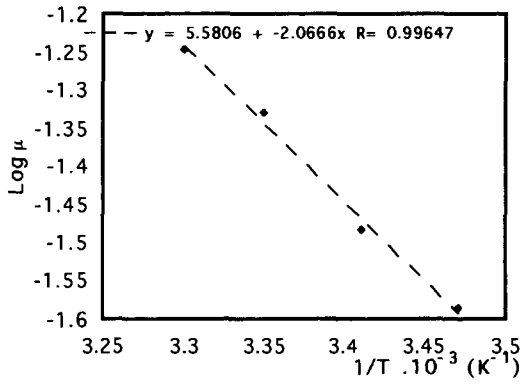

Fig. 3. Plot log μ vs. $1/T$ from which the activation energy, E_a, can be obtained.

namely

$$k = A \exp(-E_a/RT) \quad (1)$$

where k is the rate constant, A the so-called pre-exponential factor, R the gas constant and T the absolute temperature. It is assumed that the pattern of bacterial growth is similar to the transition-state theory of chemical process; thus, the activation energy corresponding to the growing process can be calculated from experimental data through an Arrhenius-type equation

$$\log \mu = \frac{-E_a}{2303RT} + C \quad (2)$$

where C is a constant.

From the plot of log μ vs. $1/T$ (Fig. 3) a value $E_a = 39.57$ kj mol^{-1} was obtained. This value is of the same order as those obtained by different authors for biochemical processes [5,9,10].

The effect of temperature variation on the degradation of microbial activity in soil can also be studied using the absolute reaction-rate theory. The rate constant is taken as the rate constant corresponding to microbial degradation in soil.

A knowledge of the apparent Gibbs energy of activation is of interest in order to interpret the microbial activity for the degradation of glucose and the degradation activity for glucose in soil.

To calculate the Gibbs free energy changes associated with this process, it is necessary to derive an equation based on the following assumptions:

1. The substrate spreads and actively reacts on the microorganism surface.

2. The microorganism absorbs the substrate and forms a bacterium–substrate complex.
3. This complex splits to produce a new organism and a metabolic substance.

The scheme of the whole process is

$$V + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [VS] \xrightarrow{\mu} 2V + P \tag{3}$$

where V is a viable cell, S the substrate, $[VS]$ the microorganism–substrate complex, P the product and μ the microbial growth rate constant.

$$V + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [VS] \tag{4}$$

According to this pattern, the first step of microbial growth is formation of the complex $[VS]$, and the reaction soon reaches equilibrium.

The equilibrium constant, $K^{\neq}$, for the formation of this transition is obtained from Eq. (4) as follows [10]:

$$K^{\neq} = \frac{[VS]}{[V][S]} \tag{5}$$

where $[VS]$ = concentration of the transient complex, $[V]$ = concentration of viable cells, and $[S]$ = concentration of the substrate.

It has been shown from the absolute reaction-rate theory that

$$\mu[V][S] = [VS]\frac{kT}{h} \tag{6}$$

where k is the Boltzmann constant, h the Plank's constant and T the absolute temperature.

From Eqs. (5) and (6)

$$\mu \equiv \frac{kT}{h}\frac{[VS]}{[V][S]} = \frac{kT}{h}K^{\neq} \tag{7}$$

and

$$K^{\neq} = \frac{\mu h}{kT} \tag{8}$$

The equilibrium constant, $K^{\neq}$, is related, on the other hand, to the free energy change, $\Delta G^{\neq}$, as follows [11,12]:

$$\Delta G^{\neq} \equiv -RT \ln K^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq} \tag{9}$$

where $\Delta H^{\neq}$ is the heat of reaction of activation, $\Delta S^{\neq}$ the entropy change of activation and R the gas constant.

Table 3
Values of $\Delta G^{\neq}$ at various temperatures

T/K	$\Delta G^{\neq}$/kJ mol^{-1}
288.15	98.85 ± 0.44
293.15	100.03 ± 0.22
298.15	100.92 ± 0.29
303.15	102.40 ± 0.17

From Eqs. (8) and (9)

$$\Delta G^{\neq} \equiv RT \ln \frac{kT}{\mu h} = RT\left(\ln \frac{kT}{h} - \ln \mu\right) \tag{10}$$

Table 3 shows values of changes in Gibbs free energy at various temperatures. These values correspond to the transition state, not necessarily the same as the viable cell–substrate complex. It can be seen that all these values are positive, thus indicating the impossibility of these processes to proceed spontaneously. Also, changes in Gibbs free energy increase with temperature, which means the increasing instability of the different transition states, making their splitting easier and increasing microbial activity.

The dependence of Gibbs free energy changes on temperature is shown in Fig. 4.

From the values determined for the activation energy and the changes in Gibbs free energy, the changes in activation entropy, $\Delta S^{\neq}$, at different temperatures were calculated using the following equation:

$$\Delta G^{\neq} \equiv \Delta H^{\neq} - T\Delta S^{\neq} = E_a - T\Delta S^{\neq} \tag{11}$$

An error, due to the fact that $\Delta H^{\neq}$ in Eq. (11) is made approximately equal to E_a, must be accounted

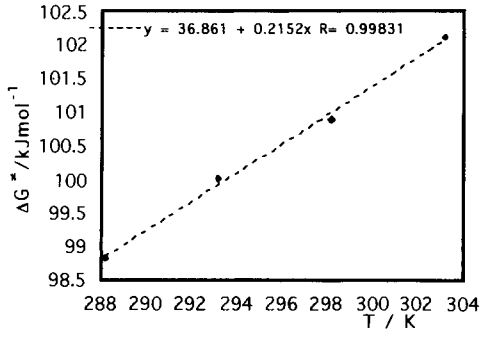

Fig. 4. Correlation between $\Delta G^{\neq}$ and temperature.

Table 4
Values of $\Delta S^{\neq}$ at various temperatures calculated from the equation $\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$

T/K	$\Delta S^{\neq}$ /J mol^{-1} K^{-1}
288.15	−205.71 ± 1.52
293.15	−206.25 ± 0.72
298.15	−205.77 ± 1.00
303.15	−206.39 ± 0.57

for. However, this error is not necessarily grave because, in most biological systems, in the temperature range studied, $\Delta H \gg RT$. Values of $\Delta S^{\neq}$ are given in Table 4. It can be seen that, in the temperature range studied, changes in the activation entropy are almost temperature-independent. To study the effect of temperature on ecological systems is very important, because most parts of microorganisms are very sensitive to temperature changes, and discussions based on data that have been collected at fixed temperature become less meaningful. These studies may be done using the microcalorimetric technique which proves to be a valuable tool in studying complex ecosystems.

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References

[1] L. Núnez, N. Barros, I. Barja, Thermochim. Acta, 237a (1994) 73.
[2] L. Nunez, N. Barros, I. Barja, J. Therm. Anal., 41b (1994) 1379.
[3] T. Kawabata, H. Yamano, T. Takahashi, Agric. Biol. Chem., 47 (1983) 1281.
[4] G.P. Sparling, Soil. Biol. Chem., 13 (1981) 153.
[5] H. Yamano, K. Takahashi, Agric. Biol. and Chem., 47 (1983) 1493.
[6] S.C. Nichols, A.M. James, Microbios, 31 (1981) 153.
[7] S.A.M. Critter, J.A. Simoni, C. Airoldi, Thermochim. Acta, 232 (1994) 145.
[8] J. Suurkuusk, I. Wadsö, Chem. Scripta, 20 (1982) 155.
[9] E.H. Battley, Energetics of Microbial Growth, John Wiley and Sons. New York, 1987, p. 255.
[10] S. Itoh, K. Takahashi, Agric. Biol. Chem., 48 (1981) 271.
[11] W.H. Xie, C.L. Xie, S.S. Qu, T.Q. Yan, Thermochim. Acta, 195 (1992) 297.
[12] S. Aiba and A.E. Humphrey, Biochemical Engineering, Millis-2nd edn., Academic Press, New York. London, 1973, p. 103.