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A kinetic analysis of the degradation of glucose by soil microorganisms studied by microcalorimetry

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Abstract

Microcalorimetry was used to study the microbial degradation of glucose in soil. Relationships between heat evolution and the viable cell counts permit the quantification of kinetic parameters for microbial growth in soil, such as Monod's substrate constant, $K_s = 1.62 \pm 0.08$ mM, and the maximum microbial growth rate constant, $\mu_{max} = 0.26 \pm 0.01\ h^{-1}$.

INTRODUCTION

Considerable attention has been given to the kinetics of bacterial growth in pure cultures. The most common patterns studied by bacteriologists are logarithmic or exponential kinetics.

Microcalorimetry is one 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 [1–4] but calorimetry has seldom been applied to investigations of microbial growth in soils [5–7]. Microcalorimetry has the advantages 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 from the catabolic breakdown of substrate, anabolic reactions contributing little to the overall balance [8]. Microcalorimetry is, therefore, a suitable technique with which to assess the overall catabolism of diverse organisms in a complex medium such as soil.

In this work, we have used microcalorimetry to study the kinetics of glucose degradation by soil microbes and its relationship with the rate of heat production and microbial growth.

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TABLE 1

Soil characteristics

Water-holding capacity:	24.33%	C/N:	15%
Percentage of carbon:	10.27%	pH:	4
Percentage of nitrogen:	0.69%	Water content:	30%

EXPERIMENTAL

Soil samples were obtained from a forest in "El Pedroso" (Santiago de Compostela, Spain). Some of their properties are listed in Table 1. The sampling was made from about 10 randomly chosen points from each site. After the removal of the very top layer of soil, samples were collected to a depth of about 15 cm. All samples from one site were mixed and sieved (mesh size $2 \times 2\ mm^2$) and the water content, organic matter and pH were measured. The bulk sample was then kept in a closed polyethene bag at 4°C for up to 3 months. Such a treatment ensures satisfactory reproducibility of the measurements [7].

The calorimetric system was a 2277 Thermal Activity Monitor (Thermometric AB, Sweden), which is a commercial version of the one developed by Suurkuusk and Wadsö [9]. Experiments were performed in hermetically sealed 5-ml stainless steel ampoules. For microcalorimetric measurements, 0.1 ml of a nutrient solution containing 1.25 mg of glucose was added to 1 g of soil sample. The reference ampoule was filled with 1 ml of distilled water. All experiments were carried out at 35°C.

The number of living bacteria was counted by the most probable number method.

The decrease in the amount of glucose due to microbial degradation was followed by chemical analyses of soil samples incubated under the same conditions as those used for the calorimetric measurements. After incubation for appropriate times, the remaining undegraded glucose was extracted from the soil by adding an excess of distilled water.

The soil suspension was then filtered and samples of filtrate were taken for the determinations of glucose. These measurements were performed by a Hitachi 747 autoanalyser, in the Hospital General de Galicia. The reactive used was glucose hexokinase (Boehringer-Manheim).

RESULTS

Figure 1 shows the power–time curve recorded from soil samples. Taking as reference the baseline of 1 g of soil without nutrient solution, there is an increase in heat flow 5 h after the addition of glucose. The activity goes on for about 15 h, after which a strong inhibition is observed and the power–time curve returns to the baseline.

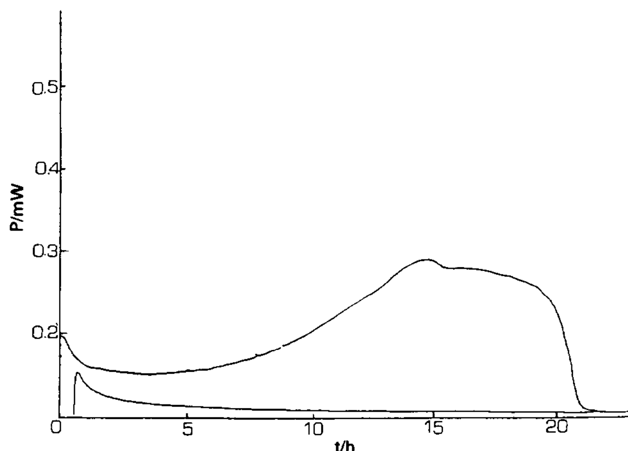

Fig. 1. Power–time curve recorded for 1 g of soil.

For further application of microcalorimetry to quantitative studies of soil microbes, it was necessary to establish the relationship between heat evolution associated with glucose degradation and the activity of microbes in soil. The number of microorganisms in the soil samples were counted before the addition of glucose ($t = 0, N_0$) and at the time at which the power–time curve reached the maximum peak-height ($t = 15$ h, N_{15}). An increase was found, from $N_0 = 0.77 \times 10^6$ to $N_{15} = 2.45 \times 10^8$ microorganisms per gram of dry soil.

From the equation

$$\log N_t - \log N_0 = \mu(t - t_0) \quad (1)$$

the microbial growth rate constant μ was calculated: $\mu = 0.16\ \mathrm{h}^{-1}$.

During microbial growth, the heat flow rate increases exponentially [10] and, thus, the specific growth rate constant μ may be determined from the slope of the straight lines obtained by semi-logarithmic conversion of the heat flow rate (Fig. 2): $\mu = 0.15 \pm 0.01\ \mathrm{h}^{-1}$.

This value is very close to that derived from eqn. (1). This similarity permits microbial growth curves to be obtained using the microcalorimetric method, taking the initial number of microorganisms N_0 as a reference.

The amount of glucose consumed ($S_0 - S_t$) by the soil microorganisms was determined by means of chemical analysis. The results are shown in Table 2. It was found that at point A of Fig. 3, the concentration of glucose had decreased from 1.25 to 0.03 mg ml^{-1}, and at point B, glucose had been completely exhausted.

In order to associate the increase in the amount of viable biomass, which grows by consuming glucose as an energy source, with the heat evolution observed during the incubation of soil with glucose, the number of viable cells ($N_t - N_0$), and the amount of glucose consumed ($S_0 - S_t$) were plotted

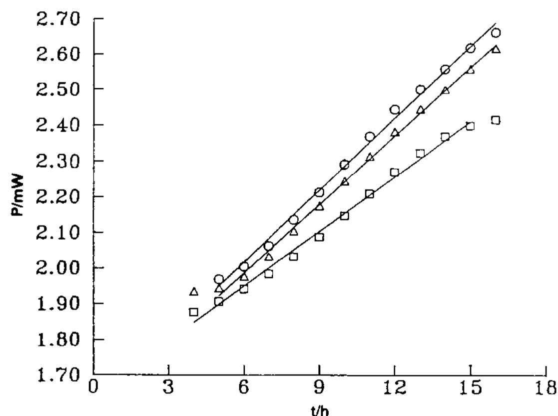

Fig. 2. Log P versus time plot obtained from the semi-logarithmic conversion of power–time curves recorded from soil samples containing glucose.

against heat evolution Q_t. As we show in Figs. 4 and 5, straight lines were obtained. The average heat evolution for the formation of a unit cell q can be obtained from the slope of Fig. 4: $q = 0.22 \times 10^{-8}$ J per cell.

From the slope of Fig. 5, the average heat evolution per unit glucose degraded α was also calculated: $\alpha = 1.446.51$ kJ per mol glucose.

Values of α and q give an apparent yield constant Y_g for the formation of microbial cells [11] $Y_g = \alpha/q = 0.66 \times 10^{14}$ cells per mol glucose.

DISCUSSION

Because heat evolution is proportional to the amount of glucose degraded and also to the increase in viable biomass, the value of μ reported can reasonably be regarded as the specific degradation rate of glucose and may be used as an index term to express how fast the material is decomposed by microbial action. If the same method is applied to the study of other organic

TABLE 2

Values of the amount of glucose degraded by soil microbes ($S_0 - S_t$) determined by chemical analysis at different times

Time/h	Q_t/J g^{-1}	$(S_0 - S_t) \times 10^{-3}$ per g glucose
0	0.30	0.00
5	0.49	0.01
10	1.43	0.14
15	3.11	0.34
20	5.54	0.27
25	7.98	0.94
40	12.41	1.44

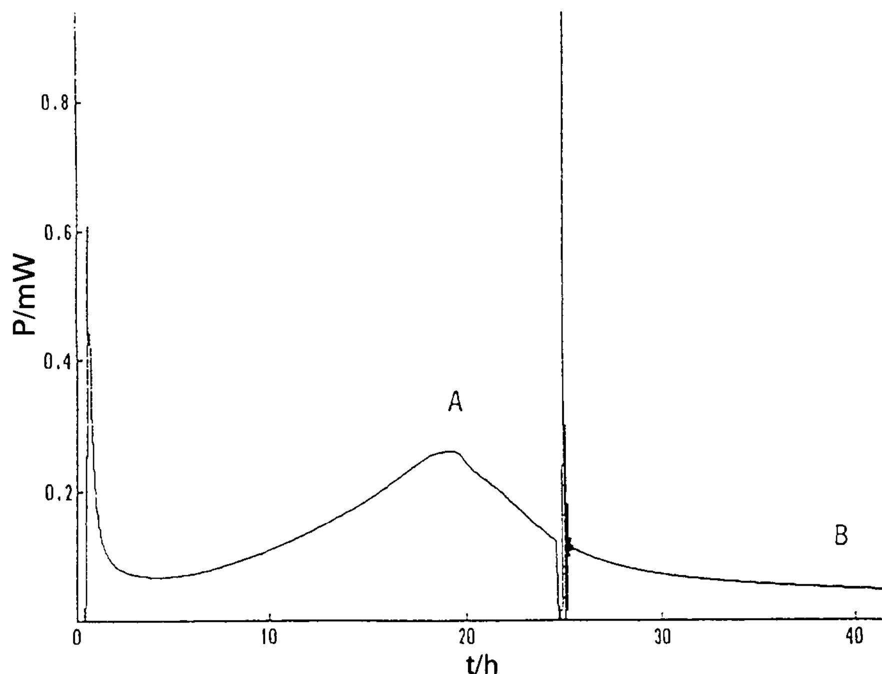

Fig. 3. The power–time curve corresponding to the heat flow rate of the soil samples. After opening the ampoule to eliminate the supposed excess of CO_2, no new increase in heat flow is observed. The fall of the power–time curve to the baseline is due to the exhaustion of glucose.

substances, information about microbial degradability, which is very important in ecology, will be obtained [11].

The value of α reported in this paper is about half of the heat combustion of glucose, $\Delta_c H = -2800$ kJ per mol glucose, and a little higher than the value $\alpha = 1287$ kJ per mol glucose reported by Kimura and Takahashi [11] for soils. However, it is much larger than the values of $\alpha = 90-120$ kJ per mol glucose reported for the heat evolution associated with the anaerobic growth of *S. cerevisae* [3]. The difference between the above values of α and $\Delta_c H$ is most probably due to the enthalpy changes associated with the anabolic processes, as reported for the growth of *E. coli* by Dermoun and Belaich [12, 13].

The value of $q = 0.22 \times 10^{-8}$ J per cell is very similar to that reported by Kimura and Takahashi [11] for soils, $q = 5.53 \times 10^{-8}$ J per cells.

The relationship found between the heat evolution of soil samples and the microbial activity permits the quantification of kinetic parameters for microbial soil activity, such as Monod's substrate constant K_s, and the maximum microbial growth rate constant, μ_{max}. These data were calculated on the basis of equations developed by different authors who established the relationship between heat evolution and Monod kinetics on pure cultures [14, 15].

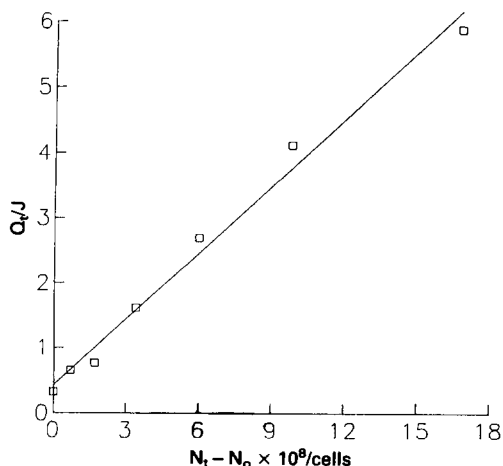

Fig. 4. Correlation between the increase in the number of microorganisms $N_t - N_0$ and the heat evolution Q_t during the incubation of the soil samples with glucose.

As we show in this paper, the heat evolution is proportional to the increase in the viable biomass; this relationship is expressed by the equation

$$Q_t = q(N_t - N_0) \quad (2)$$

The microbial growth rate constant μ is defined by

$$\mu = 1/N_t(\mathrm{d}N_t/\mathrm{d}t) \quad (3)$$

The Monod kinetic model relates the value of μ with the substrate concentration

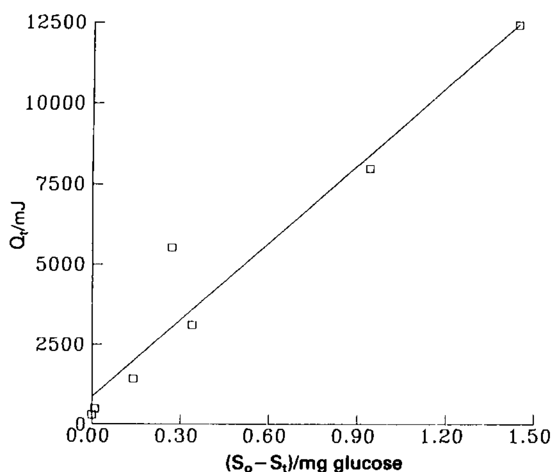

Fig. 5. Correlation between the amount of glucose consumed $S_0 - S_t$ and the heat evolution Q_t of the soil samples.

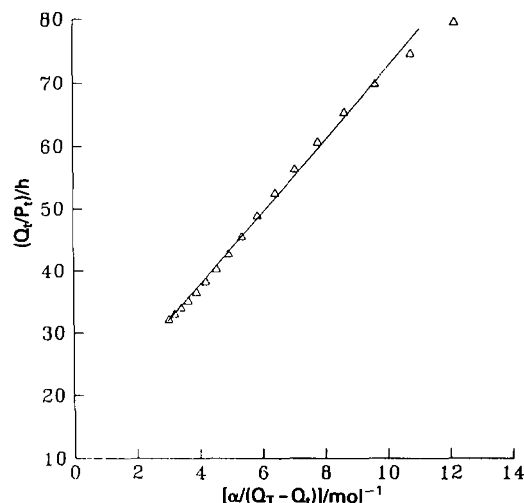

Fig. 6. Lineweaver–Burk plot of the heat evolution process during growth of soil microbes.

$$\mu = \mu_{max} S/(K_s + S) \quad (4)$$

From eqns. (2) and (3) the relationship is obtained

$$\mu = P_t/(Q_t + qN_0) \quad (5)$$

where P_t is the microcalorimetric signal output in μW.

We also confirmed that the heat evolution is proportional to the amount of glucose consumed by the soil microbes, which can be expressed by the equation

$$Q_t = \alpha(S_0 - S_t) \quad (6)$$

From the above equations, it is possible to obtain the relationship

$$P_t/Q_t + qN_0 = \mu_{max}(Q_T - Q_t)/[\alpha K_s + (Q_T - Q_t)] \quad (7)$$

where Q_T is the total heat evolution in J and Q_t is the heat evolution at time t.

As the heat evolution Q_t is generally much larger than qN_0, the above equation can be written

$$P_t/Q_t = \mu_{max}(Q_T - Q_t)/[\alpha K_s + (Q_T - Q_t)] \quad (8)$$

This last equation is a hyperbolic function; by plotting Q_t/P_t against $\alpha/(Q_T - Q_t)$, straight lines may be obtained with a slope of K_s/μ_{max}, whose intercept on the abscissa gives the value of K_s [15]. Results obtained by this method are shown in Fig. 6 and the values calculated for K_s and μ_{max} were: $K_s = 1.62 \pm 0.08$ mM; and $\mu_{max} = 0.26 \pm 0.01$ h^{-1}.

In order to compare our results for the kinetic parameters with those of other authors, Table 3 lists the different reported data for K_s and K_M. Our

TABLE 3

Values of K_M and K_s reported in the literature

Reference	Microorganism	Substrate	Conditions	T/°C	K_M/mM	K_s/mM
[16]	Yeasts	Glucose	Aerobic	30	1.8	–
[17]	Yeasts	Glucose	Aerobic	30	1	–
[18]	Yeasts	Glucose	Anaerobic	30	5.3	–
[19]	Yeasts	Glucose	Anaerobic	30	6	–
[20]	–	Glucose	Aerobic	30	2.8	–
[21]	*S. cerevisae*	Glucose	Aerobic	30	1.8	–
[15]	Yeasts	Glucose	Aerobic	30	–	1.6
[22]	Soils	Glucose	–	30	–	1.7
[23]	Soils	Glucose	–	25	2.4	–
This paper	Soils	Glucose	–	35	–	1.6

value of K_s is very close to values of K_M determined for different microorganisms in aerobic conditions and to the value of K_s for soils reported by Kumura [22]. However, it is much lower than values of K_M reported for anaerobic conditions and a little lower than the data of K_M reported by Coody et al. [23] for soils at 25°C, using a different method.

The close approach of the values of K_s and K_M strongly suggests that the external concentration of glucose is probably the factor that limits microbial growth in soil, and that this could also be kinetically limited by the transport system of the sugar [15].

We believe that the method described here can be used to characterize dynamic features of organic materials in soil and will contribute to further advances in the quantitative description of ecological systems.

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