Journal of General Microbiology (1985), **131**, 3083–3089. *Printed in Great Britain*

Calorimetric Studies of Soil Microbes: Quantitative Relation between Heat Evolution during Microbial Degradation of Glucose and Changes in Microbial Activity in Soil

By TADAHIKO KIMURA AND KATSUTADA TAKAHASHI*

Laboratory of Biophysical Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 591, Japan

(*Received 28 January 1985; revised 24 May 1985*)

Heat evolution during the microbial degradation of glucose in a brown arid andosol soil was studied in a conduction-type calorimeter at 30 °C. Reproducibilities of the degradation thermograms in terms of the peak time of thermograms, their peak heights, and the total heat evolution, were within ±0·17%, ±1·1% and ±0·51%, respectively (percentage errors). Changes in the number of viable microbial cells and in the amount of glucose degraded revealed linear relationships both between heat evolution and the amount of glucose degraded, and between heat evolution and the viable cell counts, with correlation factors of 0·987 and 0·968, respectively. The heat evolution per unit glucose was $\alpha = 1287 \pm 52$ kJ (mol glucose)$^{-1}$. The average heat effect per unit cell was $q = 6{\cdot}7$ pW per cell, consistent with values determined for bacterial cells in pure culture. On the basis of these results, we propose a method to evaluate the rate of microbial degradation of organic substances in soil. The apparent rate constant (k_d) for microbial degradation of glucose in the soil studied was $0{\cdot}302 \pm 0{\cdot}002$ h^{-1} at 30 °C.

INTRODUCTION

Many calorimetric studies have been done on microbial systems to obtain quantitative information about their biochemical and physiological activities (Beezer *et al.*, 1977; Schaarschmidt & Brettel, 1978; Belaich, 1980; Lamprecht, 1980; Newell, 1980). One of the applications of microbial calorimetry is the kinetic analysis of growth thermograms (Belaich *et al.*, 1968; Belaich & Belaich, 1976; Itoh & Takahashi, 1984). The method is quite useful, especially when applied to multimicrobial systems, in which growth thermograms corresponding to the integrated activity of multiple species are detected. There is increasing interest in the application of microcalorimetry to the study of soil microbes, since the method provides more quantitative information than other analytical devices (Konno, 1976; Ljungholm *et al.*, 1980; Kawabata *et al.*, 1983; Yamano & Takahashi, 1983). However, to extend its use, it seems essential to describe quantitatively the relationship between the heat evolution and the actual microbial activity in soils. This paper reports a quantitative study on heat evolution during the degradation of glucose by soil microbes.

METHODS

Soil. A brown arid andosol, from Tokachi, Hokkaido, Japan, was used; it was a gift from Dr T. Konno (National Institute of Agro-Environmental Science, Tsukuba, Japan).

Calorimeter. A multiplex calorimeter involving the conduction principle was used. The apparatus, designed in this laboratory, has six-membered calorimetric units, and six soil samples can be studied simultaneously. Further details of the calorimeter and its operation have been described elsewhere (Kawabata *et al.*, 1983).

Procedures. Sieved soil (10 mm × 10 mm mesh) that had been equilibrated for three months in a polyethylene bag at room temperature was used as a stock sample. The water content of the stock soil was 12·1% of the dry weight and the water capacity was 85·4% of the dry weight. To 5 g of the soil sample placed in the calorimetric

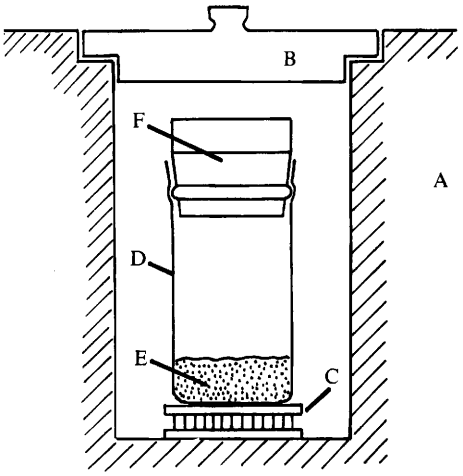

Fig. 1. Schematic drawing of the calorimetric unit. A, aluminium heat sink; B, aluminium lid; C, semiconducting thermopile plate (Sanyo TM-1012); D, glass calorimetric vessel (30 ml in volume); E, soil sample; F, polyethylene plug.

vessels was added 2 ml of nutrient solution containing 5 mg glucose and 5 mg $(NH_4)_2SO_4$. Each vial, volume 30 ml, was plugged with a polyethylene plug to prevent evaporation of water. The six glass vials containing the soil samples were placed in the calorimetric units within 3 min, and the calorimeter output signals were recorded for about 30 h. The structure of the calorimetric unit with sample vessel is shown schematically in Fig. 1. The oxygen in the air gap in the vessel was theoretically sufficient for the complete oxidative degradation of the added glucose. Measurements were made at 30 °C. The pH of the soil was 5·2; it did not change when the nutrient solution was added.

RESULTS AND DISCUSSION

To check the reproducibility of the calorimeter signals, six calorimetric recordings made for the same soil preparations containing the same amount of glucose and ammonium sulphate were compared. Fig. 2 shows the degradation thermograms obtained for 5 g soil containing 5 mg glucose and 5 mg $(NH_4)_2SO_4$. Despite the recommendation by the Interunion Commission on Biothermodynamics (1982), we have used 'μV' as the unit of the calorimeter output signal instead of the power unit 'μW', since the time integral of the apparent power–time curve is not a direct measure of the heat evolution under the experimental conditions we used. The effective instrumental time constant of the calorimetric units used was about 6 min; this required a correction to be made in order to estimate the amount of heat evolved during a given incubation time. The time course of heat evolution was calculated on the basis of equation (1) as described below according to the theory of Ono *et al.* (1965). As shown in Fig. 2, the reproducibility of the thermograms was excellent: they all had the same shape, characteristic of microbial growth thermograms (Takahashi, 1973; Beezer *et al.*, 1977; Newell, 1980). To illustrate this reproducibility more quantitatively, the peak time of the thermogram (the time at which the calorimeter signal reaches maximum amplitude), the peak height, and the total area under the thermogram, which corresponds to the total heat evolution during the degradation of glucose, were estimated from each recording and compared. The errors for the peak time (16·85 h; SD ± 0·07), the peak height (52·77 μV; SD ± 1·46) and the thermogram area (total heat evolution) (48·39 J; SD ± 0·61) were ± 0·17%, ± 1·1% and ± 0·51%, respectively. These small errors confirm the reproducibility of the thermograms observable with such a microbial system. This method is therefore applicable for the study of soil microbes and would provide detailed information about their biological activities, if appropriate analysis were made.

For the further application of microcalorimetry to quantitative studies of soil microbes, it is necessary to establish the relationship between the heat evolution associated with glucose

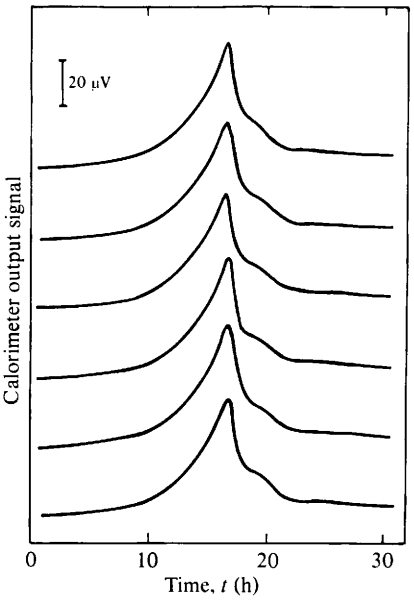

Fig. 2. Reproducibility experiment for degradation thermograms of D-glucose in brown arid andosol at 30 °C. 2 ml nutrient solution containing 5 mg glucose and 5 mg $(NH_4)_2SO_4$ was added to 5 g of the soil. The measurement was on six specimens under set experimental conditions. The thermograms shown are shifted vertically by about 25 μV on the voltage-recorder scale so that they may be easily distinguished. The calorimeter sensitivity under the steady heat effect is $A = 43{\cdot}74\ \mu W\ \mu V^{-1}$. The instrumental time constant (the reciprocal of the heat leakage modulus) is $\tau = 5{\cdot}91$ min. The conversion parameter is $\beta = A\tau = 15{\cdot}51\ mJ\ \mu V^{-1}$.

degradation and the actual activity of microbes in soil. The amount of viable biomass during incubation of the same soil preparations was measured at appropriate times by the most probable number method (Postgate, 1969). At the same time the decrease in the amount of glucose due to microbial degradation during incubation was followed by chemical analysis using the modified Somogyi–Nelson method (Hiromi *et al.*, 1963). The analyses were made on soil samples incubated under the same conditions as those used for the calorimetric measurement. 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 determination of glucose. Filtrate prepared from the soil sample without the addition of glucose was used as a blank to account for the reducing power resulting from the organic substances originally present in the soil.

The change in the number of microbial cells is shown in Fig. 3(*a*). N_0 and $N(t)$ denote the numbers of microbial cells at the start of the incubation and at time t, respectively. The plot is a typical sigmoidal curve which is characteristic of the growth of microbial cultures (Monod, 1949). Stationary phase was reached after incubation for about 20 h; the number of cells was then about 7×10^8, which was about 100 times more than at the start of incubation. Fig. 3(*b*) shows the time course of glucose degradation during incubation, which was also sigmoidal. S_0 is the initial amount of glucose (5 mg) and $S(t)$ the amount of glucose remaining unreacted in the soil at time t. The heat evolution $f(t)$, was calculated by using the relationship:

$$f(t) = g(t) + K \int g(t)\, dt \qquad (1)$$

where $g(t)$ is the apparent calorimeter output signal at time t, and K the heat leakage modulus (Newton's cooling constant) of the calorimetric unit including the soil sample (Ono *et al.*, 1965; Takahashi, 1976). The value of K was $10{\cdot}16\ h^{-1}$ in the present experimental system. The time course of heat evolution thus obtained is shown in Fig. 3(*c*).

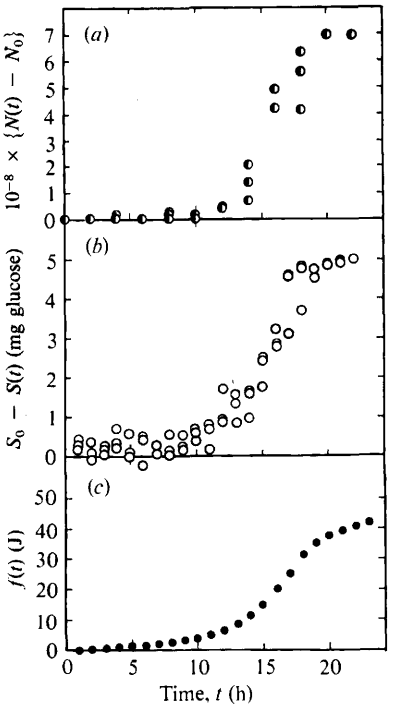

Fig. 3. Changes in (*a*) the number of viable microbial cells, (*b*) the amount of glucose degraded and (*c*) the heat evolution during incubation of brown arid andosol (5 g) with glucose (5 mg) at 30 °C.

From Fig. 3, it is clear that the changes in these quantities are very similar, strongly suggesting that the heat evolution observed during incubation of the soil with glucose is associated with the increase in the amount of viable biomass that grows by consuming glucose as an energy source. This point becomes clear if the increase in the numbers of viable cells ($N(t) - N_0$) and the amount of glucose consumed ($S_0 - S(t)$) are plotted against the heat evolution, $f(t)$ (Fig. 4*a*, *b*). From the linear relationships found, we conclude that the heat evolution observed during the degradation of glucose in soil reflects changes in the activity of viable biomass.

The average heat evolution for the formation of a unit cell was obtained from the slope of Fig. 4(*a*) as

$$\omega = (5{\cdot}53 \pm 0{\cdot}51) \times 10^{-8} \text{ J per cell} \tag{2}$$

Since, as described below, the average doubling time (t_d) of microbes in the soil studied was 2·3 h, the average heat evolution rate (the heat effect) per unit cell is calculated to be

$$\begin{aligned} q &= 5{\cdot}53 \times 10^{-8}/2{\cdot}3 \text{ J h}^{-1} \text{ per cell} \\ &= 6{\cdot}68 \text{ pW per cell} \end{aligned} \tag{3}$$

This value is very close to those determined by the calorimetry of pure cultures: $1{\cdot}8 \times 10^{-9}$ cal h^{-1} per cell (2·1 pW per cell) for *Escherichia coli* (Prat, 1969) and $3{\cdot}5 \times 10^{-9}$ cal h^{-1} per cell (4·1 pW per cell) for *Saccharomyces cerevisiae* (Lamprecht *et al.*, 1971). This again indicates that the heat evolution is a reflection of the increase in the microbial biomass in the soil studied.

From the slope of Fig. 4(*b*), the average heat evolution per unit of glucose degraded was

$$\alpha = 1287 \pm 52 \text{ kJ (mol glucose)}^{-1} \tag{4}$$

This is about one-half of the heat of combustion of glucose [$\Delta H_c = -2800$ kJ (mol glucose)$^{-1}$]. However, it is very much larger than the values of 90–120 kJ (mol glucose)$^{-1}$ reported for the

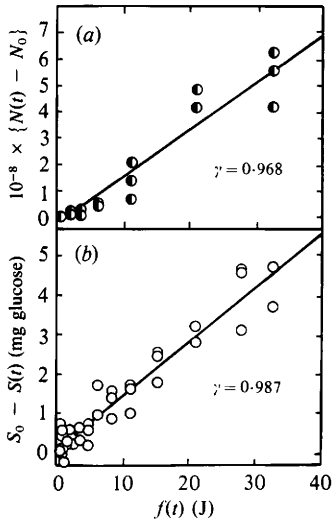

Fig. 4. Correlations between (*a*) the increase in viable biomass and the heat evolution and between (*b*) the amount of glucose degraded and the heat evolution observed during incubation of brown arid andosol (5 g) with glucose (5 mg) at 30 °C.

heat evolution associated with the anaerobic growth of *S. cerevisiae* (Lamprecht, 1980) on a liquid synthetic medium containing glucose as the sole energy source. The difference between the above values for α and ΔH_c is most probably due to the enthalpy changes associated with the anabolic processes, as reported for the growth of *E. coli* by Dermoun & Belaich (1979, 1980).

The above two values of ω and α give an apparent yield constant for the formation of microbial cells of

$$Y_g = \alpha/\omega = 2{\cdot}33 \times 10^{13} \text{ cells (mol glucose)}^{-1} \quad (5)$$

From this value, it can be calculated that 3×10^{10} molecules of glucose are required to form the unit microbial cell in soil.

In a previous paper (Hashimoto & Takahashi, 1982), we reported that the heat evolution during the exponential growth of a microbe is given by the following equation:

$$f(t) = AN_0 \exp(\mu t) + BN_0 \quad (6)$$

where μ is the growth rate constant and A and B are constants. On the assumption that this relationship also holds for the experimental system described here, a Guggenheim plot (Takahashi, 1976; Hiromi, 1978; Hashimoto & Takahashi, 1982) was used to evaluate the 'apparent growth rate constant' of the microbes in the system (Fig. 5): the plot was linear for incubation times up to 15 h. This indicates that, even with a system involving many species of microbes, equation (6) can be used to obtain quantitative information about microbial activity in terms of kinetic parameters. From the slope of the plot, the apparent growth rate constant (μ) was $0{\cdot}302 \pm 0{\cdot}002$ h^{-1} (mean, $\pm$ SD, of the six measurements).

Another way to express microbial activity is by 'generation time', defined as the time required for cell division. This is also frequently called 'doubling time'. In the soil microbial system, where the integrated activity of many species of microbes is detected, it seems more practical and convenient to use the term doubling time, representing a definite period during which the integrated activity of the microbes in soil is doubled. The doubling time (t_d) was 2·3 h for our system.

For a growing culture of pure microbes, the growth rate constant is generally estimated from the plot of $\ln N(t)$ versus t in our notation. This plot was made with the results given in Fig. 3(*a*) and is shown in Fig. 6. The solid line was drawn assuming $\mu = 0{\cdot}302$ (determined by the

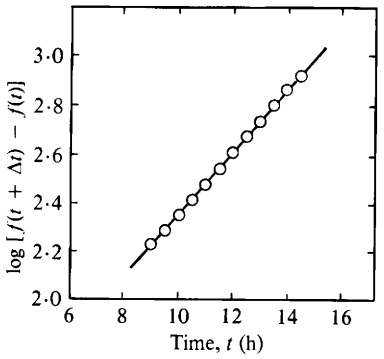

Fig. 5

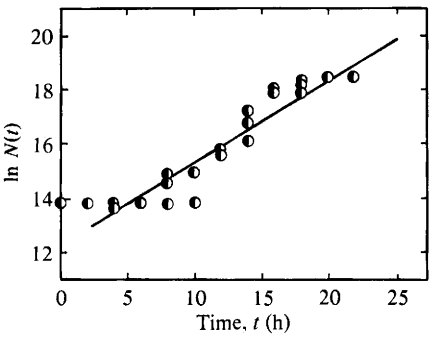

Fig. 6

Fig. 5. Guggenheim plot of the evolution of heat. The values of $f(t)$ were calculated for the observed thermogram $g(t)$ by using equation (1). The plot was made on the basis of the equation $\log [f(t + \Delta t) - f(t)] = \log AN_0(e^{\mu\Delta t} - 1) + (\mu/2{\cdot}303)t$ which was derived from equation (6). Δt in the above equation is the appropriate time interval, which is usually taken to be approximately equal to the doubling time t_d. For the plot, Δt was taken to be 2 h in the present system whereas the doubling time was 2·3 h as described in the text.

Fig. 6. Semilogarithmic plot of the changes in the numbers of viable microbial cells in 5 g brown arid andosol during incubation with 5 mg glucose at 30 °C.

Guggenheim method described above). The experimental data fall in a reasonable range within experimental uncertainty. This further supports the view that the heat evolution observed during the microbial degradation of glucose in soil is proportional to the change in the amount of viable biomass.

The value of μ determined above is only an apparent one and does not give any information about the biochemical activity of the individual organisms. However, since heat evolution is also proportional to the amount of glucose degraded (Fig. 4*b*), the kinetic parameter, determined from either $f(t)$ or $N(t)$, can reasonably be regarded as the specific degradation rate (k_d) of glucose under the given conditions in soil, and may be used as an index to express how fast the material is decomposed by microbial action. Thus

$$k_d = \mu = 0{\cdot}302 \pm 0{\cdot}002 \quad (7)$$

It is evident that this parameter is a constant indicative of the susceptibility of glucose to degradation by the soil microbes in quantitative terms. If the same method is applied to the study of other organic substances, information about microbial degradability, which is very important in ecology, will be obtained. 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.

The authors thank Dr T. Konno of the National Institute of Agro-Environmental Science, Japan, for providing us with the standard soil samples. They are also grateful to Drs K. Izumori and R. Kimura of Kagawa University, Kagawa, Japan for their kind advice in conducting the most probable number method.

REFERENCES

BEEZER, A. E., FUJITA, T., NUNOMURA, K., BRETTEL, R., SCHAARSCHMIDT, B., ZOTIN, A. I., LAMPRECHT, I., MONK, P., FORREST, W. & WADSÖ, I. (1977). Microbiological calorimetry. In *Application of Calorimetry in Life Sciences*, pp. 107–156. Edited by I. Lamprecht & B. Schaarschmidt. Berlin: Walter de Gruyter.

BELAICH, A. & BELAICH, J. P. (1976). Microcalorimetric study of the anaerobic growth of *Escherichia coli*: measurement of the affinity of whole cells for various energy substrates. *Journal of Bacteriology* **125**, 19–24.

BELAICH, J. P. (1980). Growth and metabolism in bacteria. In *Biological Microcalorimetry*, pp. 1–42. Edited by A. E. Beezer. New York: Academic Press.

BELAICH, J. P., SENEZ, J. C. & MURGIER, M. (1968). Microcalorimetric study of glucose permeation in microbial cells. *Journal of Bacteriology* **95**, 1750–1757.

DERMOUN, Z. & BELAICH, J. P. (1979). Microcalorimetric study of *Escherichia coli* aerobic growth: kinetic and experimental enthalpy associated with growth on succinic acid. *Journal of Bacteriology* **140**, 377–380.

DERMOUN, Z. & BELAICH, J. P. (1980). Microcalorimetric study of *Escherichia coli* aerobic growth: theoretical aspects of growth on succinic acid. *Journal of Bacteriology* **143**, 742–746.

HASHIMOTO, M. & TAKAHASHI, K. (1982). Calorimetric studies of microbial growth: quantitative relation between growth thermograms and inoculum size. *Agricultural and Biological Chemistry* **46**, 1559–1564.

HIROMI, K. (1978). Analysis of fast enzyme reactions: transient kinetics. In *Kinetics of Fast Enzyme Reactions*, pp. 188–254. Tokyo & New York: Kodansha & Halsted Press.

HIROMI, K., TAKASAKI, Y. & ONO, S. (1963). Kinetics of hydrolysis reaction catalysed by crystalline bacterial α-amylase. III. The influence of temperature. *Bulletin of the Chemical Society of Japan* **36**, 563–569.

INTERUNION COMMISSION ON BIOTHERMODYNAMICS (1982). Calorimetric measurements on cellular systems: recommendations for measurements and presentation of results. *Pure and Applied Chemistry* **54**, 671–679.

ITOH, S. & TAKAHASHI, K. (1984). Calorimetric studies of microbial growth: kinetic analysis of growth thermograms observed for bakery yeast at various temperatures. *Agricultural and Biological Chemistry* **48**, 271–275.

KAWABATA, T., YAMANO, H. & TAKAHASHI, K. (1983). An attempt to characterize calorimetrically the inhibitory effect of foreign substances on microbial degradation of glucose in soil. *Agricultural and Biological Chemistry* **47**, 1281–1288.

KONNO, T. (1976). Application of microcalorimetry to the study of soil microorganisms. *Calorimetry and Thermal Analysis* **3**, 148–151 (in Japanese).

LAMPRECHT, I. (1980). Growth and metabolism in yeasts. In *Biological Microcalorimetry*, pp. 43–112. Edited by A. E. Beezer. New York: Academic Press.

LAMPRECHT, I., MEGGERS, C. & STEIN, W. (1971). Mikrokalorimetrische Untersuchungen zum Stoffwechsel von Hefen. *Biophysik* **8**, 45–52.

LJUNGHOLM, K., NORÉN, B. & ODHAM, G. (1980). Microcalorimetric and gas chromatographic studies of microbial activity in water leached, acid leached and restored soils. *Oikos* **34**, 98–102.

MONOD, J. (1949). The growth of bacterial cultures. *Annual Review of Microbiology* **3**, 371–394.

NEWELL, R. D. (1980). The identification and characterization of microorganisms by microcalorimetry. In *Biological Microcalorimetry*, pp. 163–186. Edited by A. E. Beezer. New York: Academic Press.

ONO, S., HIROMI, K. & TAKAHASHI, K. (1965). Calorimetric studies on hydrolysis of glucosides. I. Heats of hydrolysis of maltose and phenyl α-maltoside. *Journal of Biochemistry* **57**, 799–807.

POSTGATE, J. R. (1969). Viable counts and viability. *Methods in Microbiology* **1**, 611–628.

PRAT, H. (1969). Calorimetry of higher organisms. In *Biochemical Microcalorimetry*, pp. 181–198. Edited by H. D. Brown. New York: Academic Press.

SCHAARSCHMIDT, B. & BRETTEL, R. (1978). Heat production of living systems: the change of the function during the growth of microbial cultures. In *Thermodynamics of Biological Processes*, pp. 181–190. Edited by I. Lamprecht & A. I. Zotin. Berlin: Walter de Gruyter.

TAKAHASHI, K. (1973). Design of a calorimeter for the continuous study of heat production during anaerobic microbial growth. *Agricultural and Biological Chemistry* **37**, 2743–2747.

TAKAHASHI, K. (1976). Microcalorimetric methods. In *Seikagaku Jikken Koza* (*Experimental Methods in Biochemistry*), vol. 12, pp. 19–37. Edited by the Japanese Biochemical Society. Tokyo: Tokyo Kagaku Dojin (in Japanese).

YAMANO, H. & TAKAHASHI, K. (1983). Temperature effect on the activity of soil microbes measured from heat evolution during the degradation of several carbon sources. *Agricultural and Biological Chemistry* **47**, 1493–1499.