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# INTERPRETATION OF THE METABOLIC ENTHALPY CHANGE, $\Delta H_{met}$, CALCULATED FOR MICROBIAL GROWTH REACTIONS IN SOILS

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## Abstract

The microcalorimetric method was used to calculate the metabolic enthalpy change per mol of glucose degraded by soil microorganisms, $\Delta H_{met}$. This parameter has been calculated by microcalorimetry for many organic, inorganic and biochemical reactions, but there is only some information about its quantification for microbial growth reactions in soils. Values of $\Delta H_{met}$ were calculated for different soil samples collected in Galicia (Spain) and Campinas (São Paolo, Brazil). Exponential microbial growth was stimulated in all soil samples by the addition of glucose and power–time curves were recorded. Results showed changes in the values of $\Delta H_{met}$ calculated for all the soil samples, suggesting a dependence of this value with the microbial growth rate constant, with the percentage of growth, with the initial number of microorganisms of soil samples, with the quantity of glucose added and with the strain of bacteria growing in soil.

The interpretation of variations of $\Delta H_{met}$ provides important qualitative and quantitative information. It reports data that allow to interpret from a qualitative point of view, the increase in biomass as a consequence of the degradation of the organic matter in soil, to understand changes in the percentages of soil organic matter and to know if the microbial population growing in differential soil samples is homogeneous. Therefore, to report that value would be very important in ecological studies, but beforehand, it is necessary to solve some problems that can appear in the experiments done to make the quantification.

**Keywords:** metabolic enthalpy change, microbial soil activity, microcalorimetry

## Introduction

The enormous advantage of using the calorimetric technique is connected to the fact that the acquisition of heat changes associated with all metabolic processes involved

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in a given culture is continuously detected without disturbing the system by sampling procedures. Thus, the obtained power–time curve allows *in situ* measurements of the total microbial activity [1]. The method is quite useful when applied to multimicrobial systems such as soils, in which power–time curves, corresponding to the integral activity of multiple species, can be detected.

There is an increasing interest in the application of microcalorimetry to the study of soil microbes, since the method provides more quantitative information than other analytical devices [2–6]. The net heat change (d$Q$) during microbial growth reactions in soil can be obtained by calorimetry and is equal to the sum of the enthalpy change (d$H$) of all reactions that occur during microbial growth. Thus, even though a growth process is composed by thousands of individual metabolic reactions, the net metabolic process comprising microbial growth can be described and thermodynamically treated as a rather simple chemical reaction [7].

In this work, the thermodynamics of the microbial exponential growth reaction is interpreted for several soils by the quantification of the metabolic enthalpy change per mol of glucose degraded, $\Delta H_{met}$. Values of $\Delta H_{met}$ were calculated for different soil samples collected in Galicia (Spain) and Campinas (São Paulo, Brazil). The essential aim is to interpret and to assess the usefulness of $\Delta H_{met}$ in ecological studies.

## Material and methods

### *Soil sampling*

Soil samples were collected in Galicia (Spain) and Campinas (Brazil). Samples from Galicia correspond to a Cambisol and samples taken in Campinas correspond to a red Latosol. Sampling was carried out at about 10 randomly chosen points from each site. After the removal of the very top layer of soil, samples were collected from a depth of 5–10 cm. All samples from one site were mixed and sieved by using mesh size 2×2 mm. Water content, organic matter and pH were calculated. All samples were stored in polyethylene bags at 4°C during 3 months before calorimetric measurements.

**Table 1** Data about the percentage of humidity, percentage of soil organic matter (SOM), percentage of carbon (C), percentage of nitrogen (N) and pH of the soil samples used in this study. The percentage of carbon and nitrogen of red Latosol was not quantificated (ND)

| Soil sample | Humidity/% | SOM/% | C/% | N/% | pH |
|---|---|---|---|---|---|
| Cambisol | 30/29/15 | 12 | 10.27 | 0.69 | 4 |
| Red Latosol | 20 | 3.3 | ND | ND | 5.2 |

Soil samples collected in Galicia were brought to different humidity percentages in the laboratory. Percentages of humidity of the different soil samples are shown in Table 1 and Table 2.

**Table 2** Values of the percentage of humidity, number of microorganisms per gram of soil (No. microorg. $g^{-1}$), microbial growth rate constant, μ, percentage of growth, total heat evolution, $Q_T$, and metabolic enthalpy change per mol of glucose degraded by soil microorganisms, $\Delta H_{met}$, calculated for different samples of Cambisol collected in Galicia. Data±SD. $N$=5

| Glucose/ mg | Humidity/ % | No. microorg. $g^{-1}$ | $\mu/h^{-1}$ | Growth/ % | $Q_T$/J $g^{-1}$ | $\Delta H_{met}$/ kJ $mol^{-1}$ |
|---|---|---|---|---|---|---|
| 1.25 | 30 | $0.77 \cdot 10^6$ | 0.155±0.002 | 965 | 15.17±0.37 | –2198±75 |
| – | 29 | $8.6 \cdot 10^8$ | 0.048±0.001 | 510 | 12.74±0.64 | –1846±57 |
| – | 15 | $1.4 \cdot 10^8$ | 0.040±0.001 | 486 | 13.59±0.30 | –1969±75 |
| 12.9 | 29 | $6.1 \cdot 10^8$ | 0.072±0.003 | 1539 | 15.60±0.50 | –217±3 |

*Microbiology*

The quantity of living bacteria in soils was determined by the most probable number method and CFU (colony forming unities). Two strains of bacteria and fungi were isolated from red Latosol using specific medium cultures by means of soil extract and King medium. These strains were inoculated in steril soil, first separately and then together to quantify the $\Delta H_{met}$ values of isolated bacteria and fungi and also to study possible changes of $\Delta H_{met}$ when bacteria grow together. The results were compared to the values of $\Delta H_{met}$ calculated for the soil samples.

*Sterilized samples*

Red Latosol soil samples were treated with hydrochloric acid, having concentrations of 12.0; 5.0 and 1.0 mol $dm^{-3}$. Each sample was kept in its respective solution for 4 h, and then the solution was decanted and filtered. The soil was washed several times with distilled water to reach pH 7.0.

*Calorimetry*

The calorimetric system used was a Thermometric 2277 thermal activity monitor. This instrument is a four-channel system, in which the sample and reference are introduced simultaneously in a thermostated cylinder. Some performance specifications for this apparatus are: detection limit 0.15 mW, baseline noise <0.2 mW, detection sensitivity 0.4 $VW^{-1}$, working temperature 293–353 K, thermal stability better than $2.0 \cdot 10^{-4}$ K over a period of several days at the temperature of measurement.

Calorimetric measurements were performed in hermetically closed 5.0 $cm^3$ stainless steel ampoules. For Cambisol soil samples, base lines were recorded from 1.0 g of soil without nutrient solution in order to quantify the heat evolution of soil samples with different water contents. After these measurements, soil samples of Cambisol with different percentages of humidity were amended with 1.25 mg of glucose in a volume of 0.10 $cm^3$ of distilled water and power–time curves were recorded. The baselines were used as a blank to calculate the total heat evolution of samples amended with glucose. A volume of 1.0 $cm^3$ of distilled water was used as reference.

Red Latosol soil samples (1.50 g) were amended with a 0.8 $cm^3$ solution containing 6.0 mg of glucose and 6.0 mg of ammonium sulphate for calorimetric measurements. In this case, the reference was a sample containing 1.50 g of soil plus 0.8 $cm^3$ of water. By using this experimental procedure, it is not necessary to record the initial baseline. All calorimetric measurements were performed at 298.15±0.02 K. A blank experimental run containing glucose, ammonium sulphate and water showed no baseline deviation.

Each strain of bacteria and fungi isolated from red Lactosol were inoculated separately in steril soil and amended with the same quantity of glucose for calorimetric measurements. Power–time curves were recorded for these samples under the same experimental conditions explained above. Then, both bacteria were inoculated together in the steril soil, the same quantity of the nutrient solution defined above was added and new power–time curves were recorded for these samples.

## Results

Soil data corresponding to samples used in this study are shown in Table 1. The first data corresponds to the Cambisol sampled in Galicia during the winter. New samples were collected in the same place in spring. The percentage of humidity decreased from 30% in winter to 15% in spring. An increase in the number of microorganisms is even observed from $0.77 \cdot 10^6$ in winter to $1.4 \cdot 10^8$ in spring. Those samples collected in spring were brought to field capacity (29%) and new microorganisms counts were made for this percentage of moisture. The remainder of the samples collected in spring were kept at the humidity they had in the moment of the sampling (15%).

Figure 1 shows the power–time curves recorded from the Cambisol soil sample collected in winter amended with 1.25 mg of glucose. After a lag phase, an exponential increase of the signal is observed followed by the decline of the power–time curve to the initial baseline. At the point in which power–time curve reached its maximum value, the glucose had been totally exhausted [5].

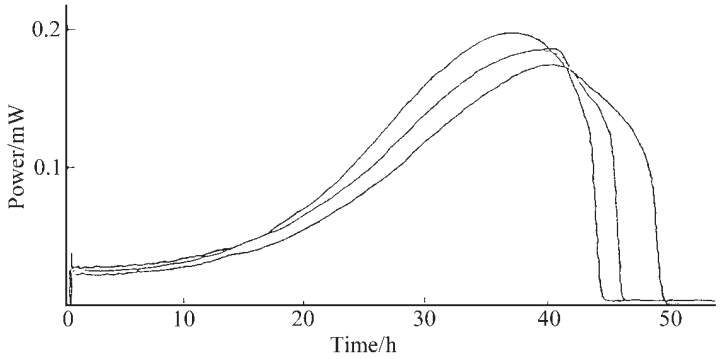


**Fig. 1** Power–time curves recorded from Cambisol soil samples collected in winter and amended with a nutrient solution containing glucose as carbon source

As the heat evolution is proportional to the quantity of nutrient added [8, 9], the metabolic enthalpy change per mol of glucose degraded by soil microorganisms can be calculated from the equation:

$$Q_t=\alpha(S_0-S_t) \quad (1)$$

where $Q_t$ is the heat evolution at a time $t$, $S_t$ is the quantity of nutrient at a time $t$, $S_0$ is the initial quantity of nutrient and $\alpha$ is the average heat evolution per unit of glucose degraded [9] which can be regarded as the metabolic enthalpy change per mol of glucose degraded by soil microorganisms, $\Delta H_{met}$. Therefore, the above equation can be rewritten as:

$$Q_t=\Delta H_{met}(S_0-S_t) \quad (2)$$

If it is assumed that glucose has been totally degraded when the power–time curve returns to the initial baseline after microbial growth, it is possible to quantify $\Delta H_{met}$ from the equation

$$\Delta H_{met}=\frac{Q_T}{S_0} \quad (3)$$

where $Q_T$ is the total heat evolution, which can be calculated from the area limited by the power–time curve recorded from soil samples [1].

Values of $\Delta H_{met}$ calculated by this method for all soil samples are shown in Table 2 together with data of the microbial growth rate constant, $\mu$, calculated also from power–time curves [10, 11], percentage of growth, total heat evolution, $Q_T$, and the initial quantity of microorganisms, No.

A clear decrease of the values of $\Delta H_{met}$ with increasing initial quantity of microorganisms, with increasing microbial growth rate constant and with increasing percentage of growth can be observed in Table 2. The sample of Cambisol sampled in winter showed the highest value of $\Delta H_{met}$, –2198 kJ mol$^{-1}$ and the highest value of the microbial growth rate constant, $\mu$=0.155 h$^{-1}$, but the initial quantity of microorganisms in this sample was very low ($0.77\cdot10^6$). Samples of Cambisol collected in spring, with percentages of humidity of 29 and 15%, show a lower value of $\Delta H_{met}$, –1846 and –1969 kJ mol$^{-1}$ respectively, than the Cambisol sample collected in winter. The microbial growth rate constant of these samples is also lower than that of Cambisol collected in winter, but the reason could be the increase of the initial number of microorganisms in these samples. Samples collected in spring with different percentages of humidity show different values of $\Delta H_{met}$. This parameter decreased from a value of –1969 kJ mol$^{-1}$, calculated for Cambisol soil sample with a percentage of humidity of 15% to –1846 kJ mol$^{-1}$ observed for the sample with a percentage of humidity of 29%. The decrease of the value of $\Delta H_{met}$ is accompanied by an increase of the microbial growth rate constant, $\mu$, from 0.040 to 0.048 h$^{-1}$, and also by an increase of the percentage of growth from 486 to 510. The power–time curves recorded from soil samples with different percentages of humidity have been previously reported [12].

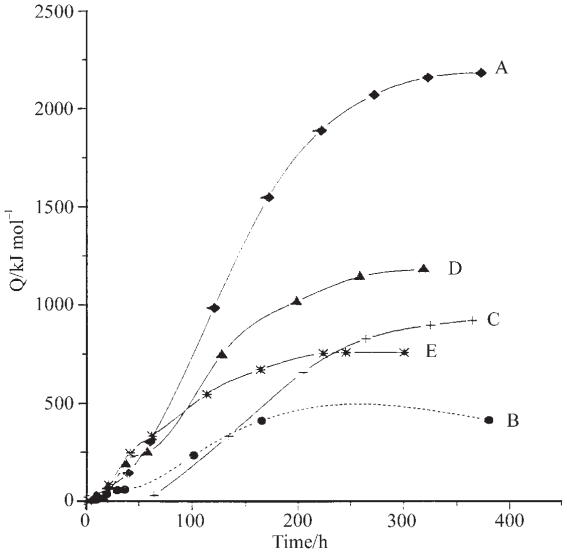


**Fig. 2** Calorimetric curves of the microbial degradation of 6.0 mg of glucose and 6.0 mg of ammonium sulphate by bacterium A – isolated in soil extract; B – isolated in B. King medium; C – by bacterium (A) and (B) growing together. Curves (D) and (E) correspond with the growth of two isolated fungi in BDA medium and inoculated in sterile red Latosol

Figure 2 shows the calorimetric curves recorded from isolated bacteria and fungi inoculated in steril red Latosol sampled in Campinas. Values of $\Delta H_{met}$ calculated for those bacteria and fungi are shown in Table 3. Both strains of bacteria growing separately show different values of $\Delta H_{met}$, –2185 and –413 kJ mol$^{-1}$. The inoculation of both bacteria together in steril soil caused a new change of the above values of $\Delta H_{met}$ to –759 kJ mol$^{-1}$. The strains of fungi inoculated in steril soil also show different values of $\Delta H_{met}$.

**Table 3** Values of the metabolic enthalpy change per mol of glucose degraded by soil microorganisms $\Delta H_{met}$ , calculated for different strains of bacteria and fungi isolated from the soil samples of red Latosol collected in Campinas

| Strains | $\Delta H_{met}$/kJ mol$^{-1}$ |
|---|---|
| Bacterium I | –2185 |
| Bacterium II | –413 |
| Bacteria I+II | –759 |
| Fungus I | –921 |
| Fungus II | –1183 |

The results listed in Table 2 even show that the increase of the quantity of glucose added to Cambisol samples at field capacity (percentage of humidity, 29%) caused a

drastic depletion of the value of the $\Delta H_{met}$ from –1846 to –217 kJ mol$^{-1}$, together with an increase of the microbial growth rate constant and percentage of growth from 0.048 to 0.072 h$^{-1}$, and from 510 to 1539 respectively. The value of $\Delta H_{met}$ did not remain constant with increasing concentration of nutrient as it should be expected.

Figures 3 to 5 show the power–time curves recorded from different samples of red Latosol collected in Campinas amended with glucose. The values of $\Delta H_{met}$ for these samples were not calculated because the limits of the integration of the power–time curves to calculate the total heat evolution, $Q_T$, are not clear. Figure 3 shows a clear inflection of the power–time curve during the exponential increase of the signal, which strongly suggests

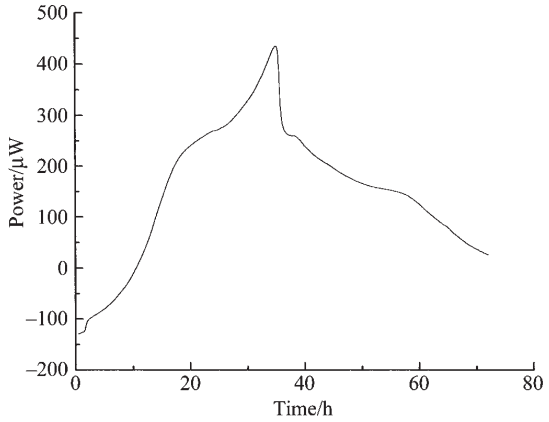


**Fig. 3** Power–time curves recorded from one sample of red Latosol amended with glucose and ammonium sulphate. It can be observed a clear inflection of the power output during the exponential increase of the signal

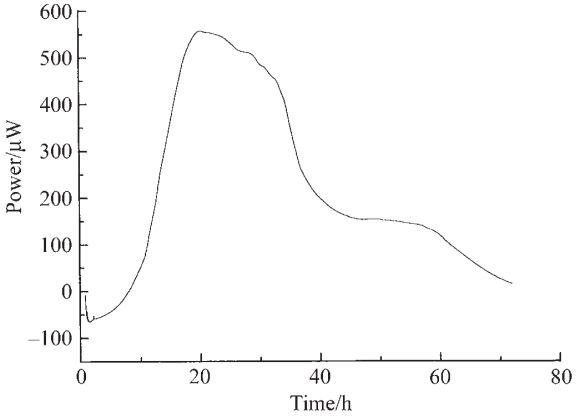


**Fig. 4** Power–time curves recorded from a new sample of red Latosol amended with glucose and ammonium sulphate. After the initial deflection of the curve to the initial baseline, a new increase of the signal is observed, affecting the limits of the integration to calculate $Q_T$

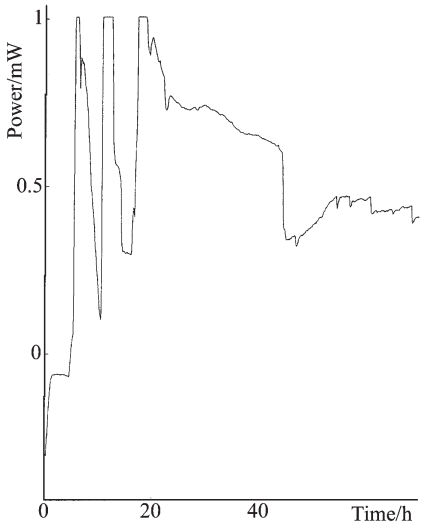


**Fig. 5** Power–time curve recorded from red Latosol under anaerobic conditions. It is observed a big instability of the signal probably due to the accumulation of gas inside the measurement ampoule

a change of the microbial growth rate constant at that point. When we calculated the microbial growth rate constant from this power–time curve, we obtained two different values: 0.157 $h^{-1}$ from the exponential increase of the signal previous to the inflection, and 0.035 $h^{-1}$ from the exponential increase after the inflection. Thus, it is not clear if glucose has been totally exhausted at the point of the inflection or at the point at which the power–time curve returns to the initial baseline.

Once again, the limits of the integration of the curve are not clear in Fig. 4. After the exponential increase of the signal, the power–time curve declines to the baseline but a new slight increase is observed. When the ampoule was opened, it was observed that a layer of water had been settled on the soil sample surface. This layer of water could have been formed by the condensation of steam from the soil, which could cause anaerobic conditions inside the ampoule during the calorimetric experiments. Therefore, the second increase of the signal observed in Fig. 4 could be due to the degradation of remaining undegraded glucose by fermentative processes. To rule this possibility out, a new quantity of nutrient solution was introduced in the ampoule to increase the layer of water. The results are shown in Fig. 5. A great instability of the signal is observed, probably due to the accumulation of gas formed by the fermentative processes inside the ampoule. The same effect should be observed in Fig. 4, if the fermentation of the glucose was the reason of the second increase of the signal.

## Discussion

Catabolic reactions by the respiratory metabolism of glucose results in an enthalpy change of –2814 kJ $mol^{-1}$ irrespective of whether energy transforming reactions oc-

cur or not. Assuming that all energy transforming intermediates are kept in a steady state, then the heat change resulting from the catabolic reaction per unit of glucose catabolically consumed is the same whether efficient coupling to anabolic reactions occurs or not. Null coupling between catabolism and anabolism because of futile cycling would give the same heat change per unit of glucose catabolically consumed as full coupling [13, 14].

In contrast, the metabolic enthalpy change per mol of glucose totally consumed by soil microorganisms is growth yield dependent. The present results show that values of $\Delta H_{met}$ decrease when the growth percentages of microbial population and when the microbial growth rate constants increase. This result strongly suggests that values of $\Delta H_{met}$ depends on the microbial growth yield in soils [15]. This behaviour is well established in studies done with isolated bacteria [16], but it had never been discussed for soil microbial activity. The results obtained from microcalorimetric experiments with isolated bacteria, growing first separately in soil and then together under the same experimental conditions, also show that values of $\Delta H_{met}$ are strain dependent.

The change in values of $\Delta H_{met}$ observed when two strains of microorganisms grow together in soil, could be due to the competition for substrates that could act in two distinct ways: either glucose is not totally degraded or the efficiency in using energy from glucose catabolism for each strain growing separately change when they grow together. It had been previously demonstrated that glucose added to soil samples used in the microcalorimetric measurements, was totally degraded at the end of the exponential curve [5]. These results are in good agreement with those obtained in similar studies [9]. Therefore, assuming that probably glucose is completely degraded in all soil samples, changes in microbial growth yield of soil microorganisms appear to be the main effect of the competition for substrate processes during microbial growth in soils. This fact could explain the changes of $\Delta H_{met}$ observed in all samples used in this study. That means, environmental conditions affect the growth yield of microorganisms and that feature causes changes in the values of $\Delta H_{met}$.

It is difficult to demonstrate that glucose is totally degraded in all soil samples by the microcalorimetric method exclusively. Some analytical techniques are necessary to support the results. Microbiological methodologies showed that at the end of the exponential growth of some isolated microorganisms, 50% of the total glucose added to the samples in the study had been totally exhausted [17]. When $\Delta H_{met}$ values were calculated, by assuming that only the 50% of the glucose added had been degraded, the obtained values ranged from –4000 to –3000 kJ mol$^{-1}$, with the exception of the Cambisol soil sample amended with 12.9 mg of glucose, which showed a value of –400 kJ mol$^{-1}$. The above values are much higher than the value of the enthalpy of combustion of glucose, –2814 kJ mol$^{-1}$. Therefore, to assume that glucose is totally degraded in the soil samples used in this study is reasonable, since otherwise the results would become very incoherent. Nevertheless, Cambisol amended with 12.9 mg of glucose shows a $\Delta H_{met}$ value of –400 kJ mol$^{-1}$, assuming only 50% of the glucose added is degraded at the end of the exponential growth. The reason could be, on the one hand, the increase of the microbial growth rate constant, $\mu$, and on the other hand, the possible incomplete degradation of the glucose in this sample if the quantity of

glucose added was too large. Therefore, it is uncertain if the $\Delta H_{met}$ value calculated for the sample of Cambisol corresponds with the real value of that parameter. A proportional increase of the total heat evolution, $Q_T$, should have been obtained to keep the value of $\Delta H_{met}$ constant with increasing quantities of nutrient [18]. The results obtained suggest that the values of $\Delta H_{met}$ in soils could be also substrate dependent.

It was observed that the shape of power–time curves recorded from different soil samples amended with glucose, can vary a great deal as it is shown in Figs 3 and 4. The inflection of the exponential increase of the signal observed in Fig. 3 and the change of the value of the microbial growth rate constant from 0.157 to 0.035 $h^{-1}$ calculated from the exponential increase of the signal in this curve, strongly suggests a diauxic behaviour of the microorganisms growing in this sample. Diauxic behaviour of microorganisms is well known, it takes place when one microorganism grows in a medium with two energy sources. The addition of ammonium sulphate, together with the glucose, to these samples provides the nitrogen and sulfur microorganism need to synthesize aminoacids. It is possible that some chemoheterotrophous microorganisms have used the ammonium sulphate as an energy source when glucose became a growth limiting factor [19]. The growth of authotrophous microorganisms was not considered because they are strongly inhibited by glucose [20]. The total heat evolution, $Q_T$, was not calculated from the power–time curve recorded from this soil sample, because the limits of the integration are not clear and the quantity of heat provided by the degradation of an inorganic energy source could contribute to the overall balance.

Figure 4 represents the power–time curve recorded from another sample of red Latosol sampled in Campinas amended with glucose and ammonium sulphate too. It can be observed a depletion of the power–time curve after the exponential increase, followed by a new increase of the signal. The above pattern brings new problems to calculate the total heat evolution, $Q_T$, because the reason of the new increase of the signal is not known.

Assuming that the glucose added has been totally exhausted at the point of the first decline of the signal, two hypothesis are proposed to the second increase: a) the growth of microorganisms that use dead biomass as an energy source and b) the growth of authotrophous microorganisms that start to use the supposedly remaining undegraded ammonium sulphate as an energy source when the glucose added has been totally exhausted [21].

All these results strongly suggest that probably glucose is totally degraded by soil microorganisms during the microcalorimetric experiments. However, as shown before, that is not the only problem to quantify the total heat evolution from power–time curves, which is one of the most important steps to report the values if $\Delta H_{met}$. In a previous work [22] the $\Delta H_{met}$ value could not be calculated for some of the soil samples, because the signal did not return to the initial baseline after the exponential increase and, therefore, the limits of the integration of the power–time curves to quantify the total heat evolution, $Q_T$, were not clear.

It is very important to report this parameter in comparative studies of the microbial activity in soils for several reasons. The metabolic enthalpy change, $\Delta H_{met}$, can be divided in a catabolic ($\Delta H_c$) and an anabolic ($\Delta H_a$) part [23–25], as given by the equation:

$$\Delta H_{met}=\Delta H_c+\Delta H_a \quad (4)$$

From this equation it is possible to calculate the anabolic part of $\Delta H_{met}$, and therefore, to quantify the percentage of energy provided from glucose catabolism invested in microbial growth. A high percentage of energy invested in growth would show a high increase of microbial biomass in soil as a consequence of the degradation of the organic matter. This fact could bring problems of immobilization of nutrients for plant growth [26]. To predict this feature could be very important for agriculture. The percentage of energy invested in growth by soil microorganisms would permit to know also the quantity of carbon from organic matter which is lost as $CO_2$ and the quantity which is kept in soil as biomass as a consequence of the degradatory activity [27]. This information would be very helpful to understand the changes of the soil organic matter due to processes such as deforestation and burning. As $\Delta H_{met}$ depends on the growth yield, similar values of this parameter obtained from different soil samples, amended with the same quantity of glucose, would indicate the same kind of microorganisms growing on those samples. The whole information would be very important in ecological studies.

As shown in the present paper, microcalorimetry is a powerful technique to study microbial activity in soils. The method provides important qualitative and quantitative data. Anyway, to avoid problems in the quantification of the total heat evolution involved in the degradation of a carbon source, it is here suggested to calculate by analytical methods, the quantity of glucose remaining in soil at the end of the exponential increase of the signal or at the point in which the power–time curve returns to the initial baseline, in order to keep clear the limits of the integration of the curve. Therefore, more assays are necessary.

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## References

1 H. Yamano and K. Takahashi, Agric. Biol. Chem., 47 (1983) 1493.
2 C. Airoldi and S. A. M. Critter, Thermochim. Acta, 288 (1996) 73.
3 K. Ljungholm, B. Norén and G. Odham, Oikos, 34 (1980) 98.
4 U. Mortensen, B. Norén and I. Wadsö, Bull. Ecol. Res. Comm., 17 (1973) 189.
5 L. Nuñez, N. Barros and I. Barja, Thermochim. Acta, 237 (1994) 73.
6 G. P. Sparling, Soil Biol. Biochem., 13 (1981) 93.
7 U. von Stockar and I. Marison, Adv. Biochem. Eng. Biotechnol., 40 (0989) 93.
8 M. Murgier and J. P. Belaich, J. Bacteriol., 105 (1971) 573.
9 T. Kimura and K. Takahashi, J. Gen. Microbiol., 131 (1985) 3083.
10 M. Hashimoto and K. Takahashi, Agric. Biol. Biochem., 46 (1982) 1559.
11 X. Wei-Hong, X. Chang tie, Q. Song-Shung and Y. Tian-Quan, Thermochim. Acta, 195 (1992) 297.
12 N. Barros, I. Gómez Orellana, S. Feijóo and R. Balsa, Thermochim Acta, 249 (1995) 161.

13 E. Gnaiger, J. Exp. Zool., 228 (1983) 471.
14 E. Gnaiger and R. B. Kemp, Biochim. Biophys. Acta, 1016 (1990) 328.
15 L. Yerushalmi and B. Volesky, Biotechnol. Bioeng., 23 (1981) 2373.
16 L. Gustafsson, Thermochim. Acta, 193 (1991) 145.
17 A. F. Gaudy, P. Y. Yang, R. Bustamante and E. T. Gaudy, Biotechnol. Bioeng., 15 (1973) 589.
18 S. A. M. Critter, J. A. Simoni and C. Airoldi, Thermochim. Acta, 232 (1994) 145.
19 W. J. Payne, Ann. Rev. Microbiol., 24 (1970) 17.
20 D. T. Brook and M. T. Madigan, Biology of Microorganisms, Prentice Hall, New Jersey 1993.
21 M. J. Pelczar and E. C. S. Chan, Elements of Microbilogy, McGraw-Hill, 1981.
22 N. Barros, S. Feijóo and R. Balsa, Thermochim. Acta, 296 (1997) 53.
23 B. Birou, I. Marison and U. von Stockar, Biotechnol. Bioeng., 30 (1987) 650.
24 L. Gustafsson, Microbes in the sea, Ellis Horwood, Chichester 1987, p. 167.
25 R. J. Winzler and J. P. Baumberger, J. Cell. Comp. Physiol., 12 (1938) 183.
26 M. Alexander, Introduction to Soil Microbiology, Wiley, New York 1961.
27 P. Prassad, S. Basu and N. Behera, Plant and Soil, 175 (1994) 85.