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Comparative study of the microbial activity in different soils by the microcalorimetric method

N. Barros, S. Feijoó*, R. Balsa

Department of Applied Physics, Faculty of Science, Campus Universitario, 15706 Santiago de Compostela, Spain

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Abstract

Microcalorimetry was applied to the study of the microbial activity of four soils with different percentages of organic matter. The qualitative study of the heat flow rate–time curves, recorded from soil samples amended with glucose, showed remarkable differences in the soil microbial activity. In order to show results in a more quantitative way, the total heat evolution, Q_{tot}, the total heat evolution of soil samples amended with glucose, $Q_{tot(glc.)}$, and the values of peak time and microbial growth rate constant, μ, were calculated from the heat flow rate–time curves recorded from all samples. Values of peak time increased with decreased microbial density and a positive correlation between total heat evolution, Q_{tot}, and percentage of organic matter, was found. Microcalorimetry appears as a suitable technique to carry out both qualitative and quantitative comparative studies of microbial activity in soils. © 1997 Elsevier Science B.V.

Keywords: Microcalorimetry; Total heat evolution; Soil organic matter; Microbial growth rate constant; Microorganisms

1. Introduction

Soil can be considered as an open system where numerous physico-chemical and biochemical reactions take place, both matter and energy being exchanged with the surroundings.

The highly heterogeneous microbial population in soils is responsible for many of the biochemical reactions, which are important for renovation and formation of soils. As the reactions due to microbial activity generate a flow of heat caused by an increase or decrease in the energy sources, microcalorimetry may be a suitable technique to study microbial activity in soils. In fact, there is increasing interest in the application of this method to study these processes [1–3]. The method has the advantage of being specific only to the initial and final energy states of a system and it is independent of organisms and reaction pathway. The heat output is derived largely from the catabolic breakdown of substrate, anabolic reactions contributing little to the overall balance [4]. Microcalorimetry also permits a continuous recording of the heat flow generated by biological reactions. Hence, the signal of a life process can be followed for long times without disturbance of the system, which is so important in studies of microbial activity in soils.

In this work, we applied the microcalorimetric method to study the microbial activity of four soils sampled in different places of Galicia, a region in the Northwest of Spain.

Soil samples were monitored calorimetrically as typical heat flow rate–time curves in order to find

*Corresponding author.

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changes in the soil microbial activity related to some properties of the soil, such as percentage of organic matter.

2. Experimental

Soil samples were collected from different sites of Galicia. Profile 1 was sampled in a private plot of land previously burned. Profile 2 corresponds to a forest soil with autochthonous vegetation, rich in organic matter. Profile 3 is a soil sample from a Pinus forest and Profile 4 was sampled in a private wine-producing land. This last one was very poor in organic matter.

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 to a depth of about 15 cm. All samples from one site were mixed and sieved (mesh size 2×2 mm.) and water content, pH, percentages of organic matter, carbon and nitrogen were determined. Results are listed in Table 1.

All soil samples were brought to field capacity humidity to avoid the influence of moisture on soil microbial activity [5]. Percentages of humidity at field capacity are also listed in Table 1.

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

The calorimetric system was a model 2277 Thermal Activity Monitor (Thermometric, AB, Sweden) which is a commercial version of the system developed by Suurkuusk and Wadsö [6]. Experiments were performed in hermetically closed 5 ml stainless steel ampoules. Base lines were recorded from 1 g of soil samples at field capacity without nutrient solution and total heat evolution, Q_{tot}, was quantified. After this treatment soil samples were amended with a quantity of glucose equivalent to 5% of the carbon content in each soil, in a volume of 0.2 ml of distilled water and heat flow rate–time curves were recorded.

All measurements were run at 25°C.

3. Results

From the areas delimited by heat flow rate–time curves, recorded from the soil samples without nutrient solution, the total heat evolution, Q_{tot}, was calculated. These values are shown in Table 2. The total heat evolution, Q_{tot}, varied with the percentage of organic matter. Fig. 1 shows a positive correlation between these two variables.

Heat flow rate–time curves recorded from soil samples amended with glucose are shown in Fig. 2. The total heat evolution, $Q_{tot(glc.)}$, was also calculated for these samples. Results are listed in Table 2. The addition of glucose induced a rapid increase of heat flow rate in all samples. All recorded heat flow rate–time curves represent a typical microbial growth. In all samples heat flow increased exponentially after a lag phase, followed by a large stationary phase in Profiles 3 and 4 and by the decline of heat flow to the initial base line in Profiles 1 and 2. As the heat flow rate increased exponentially in all soil samples amended with glucose, the microbial growth rate constant, μ, could be calculated from the semilogarithmic conversion of heat flow rate [7]. Results are shown in Table 3. It can be observed that the greatest values of microbial growth rate constant, μ, were obtained from the less organic samples.

Estimates were also made of the values of peak time (time in which the microcalorimetric signal output reaches the maximum amplitude). Results are listed in Table 3. Profile 3 showed the largest signal in mW at a peak time of 49 h. Profiles 2 and 4 showed the lowest values of peak time. The power output at the peak time

Table 1
Some properties of the soil samples used in this study

Data	Profile 1	Profile 2	Profile 3	Profile 4
C	10.27%	9%	6.35%	3.17%
N	0.69%	0.45%	0.21%	0.24%
OM	ND	15.5%	10.95%	5.47%
FCH	29%	27%	28%	23%
pH	4	4.36	3.78	5.34

C: percentage of Carbon, N: percentage of Nitrogen, OM: percentage of organic matter, FCH: Field Capacity Humidity and pH.

Table 2

Data	Profile 1	Profile 2	Profile 3	Profile 4
Q_{tot} (J g^{-1})	3.13±0.11	1.01±0.05	0.74±0.03	0.49±0.02
glc. (mg)	12.9	11.3	7.94	3.97
$Q_{tot(glc.)}$ (J g^{-1})	5.6±0.05	13.77±0.21	36.07±0.53	14.43±3.64
OM	ND	15.5%	10.95%	5.47%

Q_{tot}: Values of total heat evolution recorded from soil samples without glucose.
glc.: Quantity of glucose added to 1 g of soil sample.
$Q_{tot(glc.)}$: Values of total heat evolution of soil samples amended with glucose.
OM: percentage of organic matter.
Mean±SD, n=5

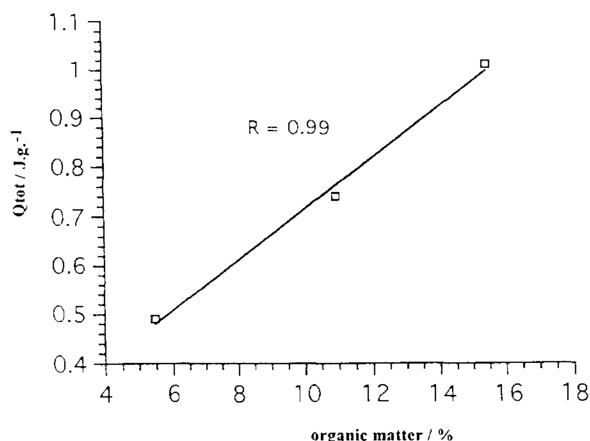

Fig. 1. Correlation between total heat evolution, Q_{tot}, calculated for soil samples without nutrient solution, and their percentage of organic matter.

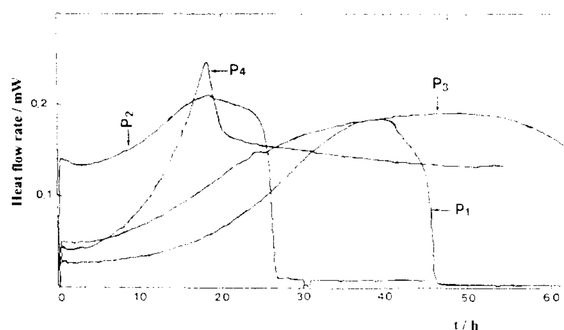

Fig. 2. Heat flow rate–time curves recorded microcalorimetrically from four soil samples with different percentages of organic matter, amended with glucose. P1: Profile 1; P2: Profile 2; P3: Profile 3 and P4: Profile 4.

and the time interval for the duration of the peak should be related to the population living in the soil samples [8].

No relation was found between the total heat evolution calculated from soil samples with and without

Table 3

Data	Profile 1	Profile 2	Profile 3	Profile 4
OM	ND	15.5%	10.95%	5.47%
Peak time	38.85±0.58	19.30±0.44	49.00±0.71	23.35±3.34
NLB per g of soil	6.1×10^{8}	190×10^{8}	48.7×10^{8}	70.5×10^{8}
μ (h^{-1})	0.072±0.003	0.037±0.001	0.038±0.001	0.109±0.012
α (kJ/mol glc.)	155.70±2.66	137.16±5.64	ND	ND

μ: Microbial growth rate constant.
Peak time in h.
NLB: Number of living bacterias per gram of soil sample.
OM: Percentage of organic matter.
α: Average heat evolution per mol glucose degraded.
Mean±SD, n=5.

glucose, and their microbial population. The most organic soil sample (Profile 2) showed the biggest number of living bacteria but Profile 4, which has the lowest organic matter percentage, also presents a high microbial density. These data can be observed in Table 3.

In Profiles 1 and 2 the heat flow rate returns to the base line after microbial growth. In a previous work [9], we reported that at the point in which the heat flow rate reaches the initial base line, after microbial growth, the glucose added had been totally exhausted by soil microorganisms. This fact permits to quantify the average heat evolution per mol glucose degraded, α, from the equation $\alpha = Q_{tot(glc.)}/S_0$, where S_0 is the initial concentration of glucose added. This parameter was calculated for Profiles 1 and 2 which followed the above kinetic. Results are listed in Table 3. Nevertheless, it was impossible to estimate, α, for Profiles 3 and 4 because the heat flow rate–time curves recorded from these samples showed a different behaviour. The exponential increase of heat flow is followed by a large stationary phase and no return to the base line was observed.

4. Discussion

Many authors are in agreement with the existence of a positive correlation between the organic matter content and the number, biomass and microbial activity in soils [9–11]. This kind of correlation has even been noted in other ecosystems such as standing crop values of bacteria in aquatic environments [12]. In this work, we report a positive correlation between the total heat evolution, Q_{tot}, quantified from heat flow rate–time curves recorded calorimetrically from soil samples without glucose, and the organic matter content of these samples. Samples with the greatest percentages of organic matter showed the greatest values of Q_{tot}, may be due to a bigger availability of substrate sensible to microbial attack. Nevertheless, no correlation between organic matter and microbial density was found.

The values of total heat evolution, $Q_{tot(glc.)}$, calculated from soil samples amended with glucose, were much higher than values of Q_{tot}. The big difference between the above parameters suggests that in these soils the metabolic activity of microorganisms is slight, possibly because part of the microbial population is inactive but also because of a low effective substrate concentrations. The addition of glucose stimulates a bigger proportion of the biomass in soil reflected in heat flow rate–time curves as a rapid increase of heat flow. Heat evolution of these samples probably proceeds from the catabolism of the glucose added [13]. The same effect was observed in studies of microbial activity in soils using the CO_2 evolution as an index of microbial activity. These changes in heat flow and CO_2 evolution suggest that the addition of a carbon source, such as glucose, to soils alters the edaphic endogenous respiration and therefore, the transformation of the organic matter pre-existent [14].

Since, heat evolution is proportional to the amount of glucose degraded by soil microorganisms and also to the increase in viable biomass [15,16], the values of microbial growth rate constant, μ, reported for the different soils used in this study, 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. The less organic sample showed the greatest value of microbial growth rate constant, which indicates that glucose was degraded faster in this profile. Except Profile 1, in which the organic matter content could not be estimated, it is the most organic sample, Profile 2, that showed the lowest value of microbial growth rate constant, which indicates a low microbial activity. This fact suggests that the organic matter in this soil suffers a slow decomposition. The reason for that may be the existence of an imbalance between the rate at which organic debris is supplied and the rate at which it is decomposed by mineralization that implies the accumulation of the organic matter in this soil. Another reason could be that the organic matter of this soil sample was in some way protected against microbial attack [17,18]. The high value of microbial growth rate constant of the less organic soil, sampled in a wine-producing land, could be explained taking into account the possibility of rapid decomposition of humus as a result of the systematic application of large accounts of mineral fertilizers not compensated by new humus formation [19]. The use of fertilizers that usually contain a high microbial population could also explain the high number of microorganisms counted in this soil sample [20,21]. The high microbial activity

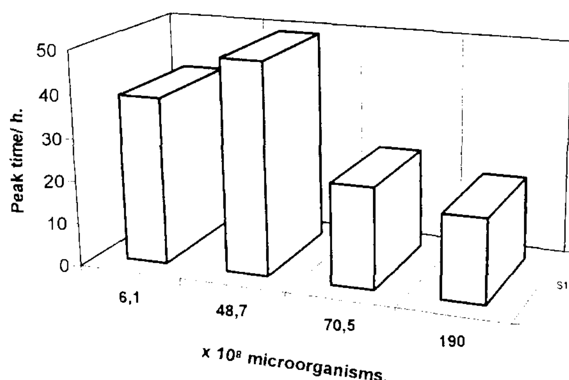

Fig. 3. Variation of the peak time with microbial density in soils. Except in Profile 1, there can be observed a clear decrease of the value of the peak time with increased microbial population in soil samples.

observed in this soil could cause problems related to immobilization of nutrients.

It was also found that the value of the peak time decreased in samples with high number of microorganisms. The relation can be observed in Fig. 3. Except in Profile 1, the durability of the peak time increased with decreased microbial population. Therefore, this relation, very common in bacterial cultures, remains in ecosystems with heterogenic populations of microorganisms such as soils [7].

The heat flow rate–time curves recorded from the soil samples amended with glucose showed remarkable differences. Profiles 1 and 2 reflect typical microbial growth in a medium limited by the carbon source. After the exponential increase of heat flow due to microbial growth, the heat flow rate–time curve declines to the base line when glucose has been totally exhausted. Nevertheless, heat flow rate–time curves recorded from Profiles 3 and 4 were always above the initial level of the base line. On one hand, it is possible that it takes a long time in these samples to consume all the glucose added. On the other hand, after the exhaustion of glucose, a portion of the biomass could remain active by decomposition of the dead microbial biomass which has the same stimulatory effect on respiration as glucose, and it is probably the most labile organic matter fraction [22]. But in that case, we think that a new exponential increase of heat flow should be observed. Anyway, the above findings suggest changes in the kinetic of glucose degradation in soil samples but more experiments are necessary for further conclusions.

Briefly, we agree with our authors [23] that the metabolic activity in these soils probably depends to a large extent on the quality and nature of soil organic matter rather than on the quantity present. More assays are necessary and microcalorimetry can be a good method for further studies.

5. Terminology

Peak time: Time in which the microcalorimetric signal output reaches its maximum amplitude

Q_{tot}: Total heat evolution in Joules per gram of soil sample

$Q_{tot(glc.)}$: Total heat evolution in Joules per gram of soil sample amended with glucose

α: Average heat evolution per mole of glucose degraded, kJ/mol.glc.

μ: Microbial growth rate constant, 1/h

glc.: Glucose

C: Carbon

N: Nitrogen

OM: Organic matter

FCH: Field capacity humidity

NLB: Number of living bacteria

References

[1] A.A. Esener, J.A. Roels and N.W.F. Kossen, Biotech. Bioeng., 25 (1983) 2803.
[2] J. Monod, Ann. Rev. Microbiol., 3 (1949) 371.
[3] J.I. Prosser and T.R.G. Gray, J. Gen. Microbiol., 102 (1977) 119.
[4] W.W. Forrest, Methods in Microbiology, Academic Press, London (1972) p. 285.
[5] N. Barros, I. Gomez-Orellana, S. Feijoo and R. Balsa, Thermochim. Acta, 249 (1995) 161.
[6] J. Suurkuusk and I. Wadsö, Chem. Scr., 20 (1982) 155.
[7] M. Hashimoto and K. Takahashi, Agric. Biol. Chem., 46 (1982) 1559.
[8] A.M. Silvana, J.A.S. Critter and C. Airoldi, Thermochim. Acta, 232 (1994) 145.
[9] P.W. Flanagan and U. Cleve, Ecol. Bull. Stockholm., 25 (1977) 261.

[10] J. Schnürer, M. Clarholm and T. Rosswall, Soil. Biol. Biochem., 17 (1985) 611.
[11] F. Azam, K.A. Malik and F. Hussain, Biol. Fertil. Soil., 2 (1986) 157.
[12] Van. Es. E.B. and L.A. Meyer-Reil, Adv. Microbiol. Ecol., 6 (1982) 111.
[13] J.P. Sparling, Soil. Biol. Biochem., 13 (1980) 93.
[14] M. Carballas, T. Carballas and F. Jacquin, An. Edafol. Agrobiol., 38 (1979) 1699.
[15] L. Nunez, N. Barros and I. Barja, Thermochim. Acta., 237 (1994) 73.
[16] T. Kimura and K. Takahashi, J. Gen. Microbiol., 131 (1985) 3083.
[17] J.K. Adu and J.M. Oades, Soil. Biol. Biochem., 10 (1978) 109.
[18] D.W. Anderson and E.A. Paul, Soil. Sci. Soc. Am. J., 48 (1984) 98.
[19] M.M. Kononova and V.V. Dokuchayev, Pochvovedemiye, 4 (1984) 6.
[20] M.S. Finstain and M.L. Morris, Adv. Appl. Microbiol., 19 (1975) 113.
[21] M. Díaz-Ravina, M.J. Acea and T. Carballas, Biol. Wastes., 30 (1989) 89.
[22] D.S. Jenkinson and J.N. Ladd, in E.A. Paul and J.N. Ladd (Eds.), Soil Biochemistry, Vol. 5, Dekker, New York (1981) p. 415.
[23] M. Díaz-Ravina, T. Carballas and M.J. Acea, Soil. Biol. Biochem., 6 (1988) 817.