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EFFECT OF STORAGE OF SOIL AT 4°C ON THE MICROBIAL ACTIVITY STUDIED BY MICROCALORIMETRY

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Abstract

The effect of the storage of soils on their microbial activity has been studied using a microcalorimetric method. Soil samples were kept in closed polyethene bags at 4°C during 3–6 months. Results show changes in the slope of the different *P–t* curves recorded from the samples stored at 4°C. This fact strongly suggest the existence of changes of the microbial activity of soil as the heat evolution is a direct measurement of the cells metabolic activity. The value of the Peak-time (time in which the microcalorimetric signal reaches the maximum value) is related with the microbial density of soil samples. This parameter was affected by the time of storage increasing with time. The total heat evolution $Q(t)$, of the soil samples amended with glucose calculated from the area limited by the Power–Time curves, also decreases with the time of storage. The soil that had been stored for 6 months before experiments, showed the lowest value of $Q(t)$.

Keywords: microbial activity, microcalorimetry

Introduction

Different factors have a marked influence on the growth process of microbes in soil. There is increasing interest in the application of microcalorimetry to the study of this process [1–3], owing to the fact that this method provides more quantitative information than other analytical devices when applied to multicomponent systems. This paper reports the effects of storage of soil at 4°C on the microbial activity in a such heterogeneous system as soil.

Experimental

All the soil samples used in our measurements were collected from a forest in Monte Pedroso (Santiago de Compostela, Spain). Some of their properties

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are listed in Table 1. The sampling was made from 10 randomly chosen points from each site to a depth of about 15 cm. Samples of each site were mixed and sieved (mesh size 2×2 mm). Water content, organic matter and *pH* of the different bulk samples were measured and after this all of them were kept in closed polyethene bags at 4°C in a refrigerator, in order to check the reproducibility of the *P–t* curves. Series of measurements were made on soil recently collected and on soils stored at 4°C for 3 and 6 months respectively.

Table 1 Soil properties

Water-holding capacity	24.33%
Carbon	10.27%
Nitrogen	0.69%
pH	4
Moisture	30.00%

Microcalorimeter was a 2277 Thermal Activity Monitor commercial version of the one developed by Suurkuusk and Wadsö [4]. All the experiments were performed using two 5 ml stainless steel hermetically closed ampoules. One of them was filled with 1 g size samples of soil amended with 0.1 ml of a nutrient solution containing 1.25 mg of glucose. Reference ampoule contained 1 ml of distilled water.

Results and discussion

Figures 1, 2 and 3 show the *P–t* curves registered for the samples recently collected and those stored at 4°C during 3 and 6 months respectively. In all the cases the addition of glucose produced a marked increase of the Thermal Power.

It was observed that the time necessary for achieving the maximum of the curves, Peak-time, increases with the time of storage. In fact, Table 2 shows Peak-time values of the fresh sample and of those stored for 3 and 6 months.

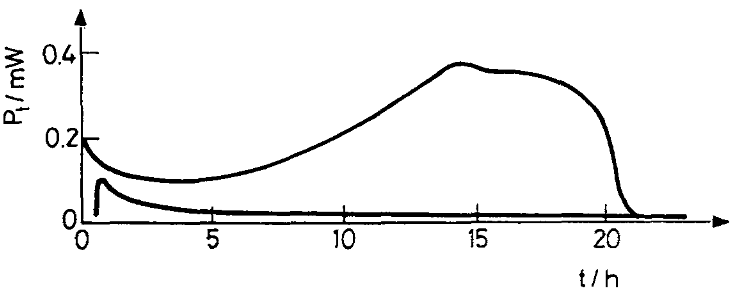

Fig. 1 *P–t* curve of a soil recently collected after being amended with glucose

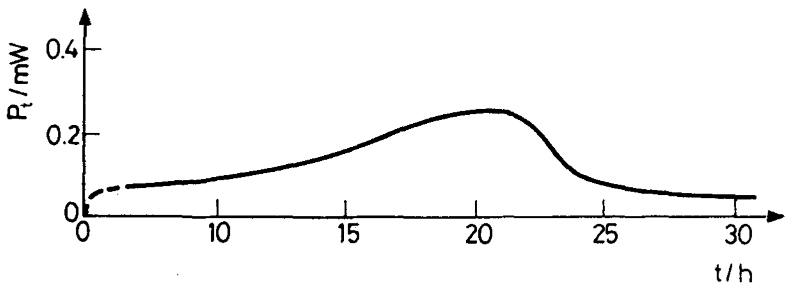

Fig. 2 *P–t* curve of a 3 months stored soil at 4°C

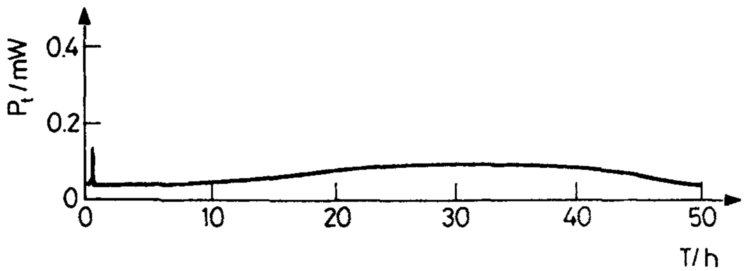

Fig. 3 *P–t* curve of a soil stored 6 months at 4°C

Table 2 Values of Peak-time, Peak-height and total heat, $Q(t)$, calculated from the curves *P–t*. The uncertainty quoted is the standard deviation of the mean

Sample	Peak-time / h	Peak-height / $J\cdot g^{-1}$	$Q(t)$ / $J\cdot g^{-1}$
Fresh soil	14.75±0.29	8.544±0.376	15.168±0.367
3 months	18.67±0.30	7.661±0.377	11.189±0.420
6 months	33.17±1.21	7.284±0.159	9.053±0.556

This values go from 14.75 h, (fresh sample), to 18.67 h (3 months) and finally to 33.17 h (6 months). At the same time, Fig. 4 shows the decrease of the total heat evolution, $Q(t)$, with the time of storage as a consequence of the decrease of the biological activity.

For all the sample, microbial growth rate constants, μ, were calculated from the slopes of the straights lines obtained when $\lg P(t)$ is plotted *vs.* time. Table 3 shows μ values. It can be observed a clear decrease of these values with storage time. This fact can be interpreted in terms of a diminution of the microbial activity.

Table 3 Values of rate constant, μ, calculated from the slope of the logarithm Thermal Power *vs*. time for the three types of soil studied

Exp.	Sample	μ / h^{-1}	Mean±SD
1	Fresh soil	0.152	
2		0.160	0.155±0.002
3		0.154	
1	3 months	0.101	
2		0.090	0.100±0.006
3		0.110	
1	6 months	0.017	
2		0.024	0.019±0.003
3		0.016	

Comparison of all the results clearly indicates that storage of soil at 4°C produces important changes of the metabolic activity of soil microorganisms. This is reflected by the decrease of both the total heat evolution, $Q(t)$, and the growth rate constant. The increase of the Peak-time with the storage time is related to a diminution of the microbial density which was checked by counting the microorganisms at appropriated times.

A similar effect was observed by analysis of the soil activity in terms of the CO_2 evolved by the samples after the addition of CO_2.

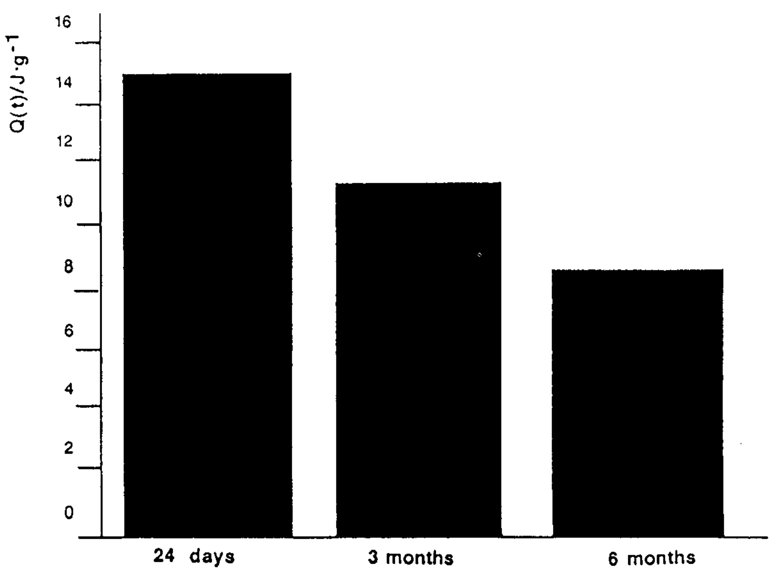

Fig. 4 Figure shows that the total heat evolution decreases with storage, at 4°C

Plots of evolved CO_2 *vs.* time are similar to *P–t* plots as the time to reach the maximum CO_2 evolution increases when the soil is stored longer than 2 months [5]. It was also observed that soil biomass decreases after being stored for more than 56 days [6].

All these facts strongly suggest that active biomass is lower in stored soil. These changes in soil metabolic activity can be detected using a microcalorimetric method [7].

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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 J. Suurkuusk and I. Wadsö, Chem. Scr., 20 (1982) 155.
5 P. C. Nannipieri, R. L. Johnson and E. A. Paul, Soil Biol. Biochem., 10 (1978) 223.
6 D. J. Ross, K. R. Tate, A. Cairns and K. F. Meyrick, Soil Biol. Biochem., 12 (1980) 369.
7 J. P. Sparling, J. Soil Sci., 34 (1983) 381.

Zusammenfassung — Mittels einer mikrokalorimetrischen Methode wurde der Einfluß der Lagerung von Böden auf ihre mikrobielle Aktivität untersucht. Bodenproben wurden bei 4°C über 3 bis 6 Monate hinweg in verschlossenen Polyethylenbeuteln gelagert. Die Ergebnisse zeigen eine Änderung des Anstieges der verschiedenen *P-t* Kurven, die von den bei 4°C gelagerten Proben aufgenommen wurden. Diese Tatsache läßt stark auf die Existenz von Veränderungen der mikrobiellen Aktivität des Bodens schließen, da die Entwicklung von Wärme eine unmittelbare Meßmöglichkeit für die metabolitische Aktivität der Zellen darstellt. Der Wert der Peak-Zeit (bei der das mikrokalorimetrische Signal sein Maximum erreicht) wird mit der Mikrobendichte der Bodenproben verglichen. Dieser Parameter ist abhängig von der Lagerungsdauer und steigt mit zunehmender Dauer an. Die anhand des von den *P-t* Kurven begrenzten Fläche berechnete totale Wärmeentwicklung $Q(t)$ der mit Glukose ergänzten Bodenproben sinkt mit zunehmender Lagerungsdauer. Bodenproben, die vor den Versuchen 6 Monate lang gelagert wurden, wiesen die geringsten $Q(t)$-Werte auf.