Journal of Thermal Analysis and Calorimetry, Vol. 87 (2007) 1, 15–19

# DEVELOPMENT OF AN EXPERIMENTAL PROCEDURE TO ANALYSE THE 'SOIL HEALTH STATE' BY MICROCALORIMETRY

*J. Rodríguez-Añón*[*]*, J. Proupín-Castiñeiras, M. Villanueva-López and O. Núñez-Fernández*

Research Group TERBIPROMAT, Department of Applied Physics, Faculty of Physics, University of Santiago de Compostela 15782 Santiago, Spain

This work is a 'historical' revision of the evolution of an experimental procedure developed by Prof. Lisardo Núñez and his research group TERBIPROMAT to study the sustainability and the soil health state.

From the very beginning, in 1993, the microbial activity was the main bioindicator selected to analyse the 'soil health state'. For this reason, a microcalorimetric technique was used lately to analyse the influence of different human activities such as reforestations, agricultural exploitation or pollution on the microbial activity in different soils. Microcalorimetry is the main scientific technique used in this research to follow the stimulation of the microbial activity by addition of glucose. The data obtained were complemented by a study of physical, chemical and biological parameters of soil and allowed to follow the microbial activity in soils of Galicia (Spain) along the year.

The final results, still in revision, will be helpful in establishing a data basis for real maps of the 'health state' of different soils. Such maps could be used to design processes that help us to decide how we should exploit soils ensuring their sustainability.

***Keywords:*** *environmental influence, microbial activity, microcalorimetry, soil health state, sustainability*

## Introduction

Soil can be defined as an open three-phase physical mean medium highly complex and heterogeneous [1, 2], dynamic, and in continuous evolution, influenced by environmental, man-made and endogenous factors.

About one third of the world agriculturally cultivable land was lost in the last 100 years, and the other part suffered a deterioration of its quality. Soil quality, or soil health, can be understood as the integration of the innate chemical, physical and biological soil attributes within a framework of space, time and land use. For this reason, soil quality is used as an indicator of soil capacity to develop its basic properties in a sustainable way, such as the environment where the active growth of plants takes place (a key for agriculture productivity), or as a water purifier system [3] (key for the distribution and the improvement of the quality of this important and limited natural resource).

For this reason, the loss of fertile land and natural resources owing to different factors produces serious problems that affect the development of the current technological society. This worrying situation forced the Food and Agriculture Organization (FAO) to declare soil an item to be protected as a support of the world society welfare. In particular, the European Union (EU) has elaborated many environmental plans focussing on the protection and maintenance of the environment under the premises of a rational and sustainable development and as a basis for the preservation of the current technological society. Since the 1980's, large stretches of fertile land traditionally used for agriculture were neglected in Galicia (NW Spain), thus originating an aggressive forest exploitation which introduced fast growing forest species to be used as raw materials for pulp production. This new 'economical' activity resulted in a stress diminishing the soil productive capacity and, as a consequence, the loss of its vegetative activity.

Therefore, and assuming the definitions of soil and soil quality, an experimental procedure was designed based on a set of specific assumptions:

- Soil quality is directly responsible for the capacity to generate and maintain agriculture performance [4].
- Soil productivity directly depends on main features that are common to every kind of soil, such as (*i*) physical fertility or capacity to supply plants with the necessary nutrients in an adequate form, proportion and time, as (*ii*) the own fertility, or as (*iii*) the supply of the water, oxygen and heat necessary for plant growth.
- Microorganisms are real bioindicators of soil quality; the soil stress originating from a continuous exploitation reduces both, the quantity and the diver-

* Author for correspondence: fabelen@usc.es

*1388–6150/$20.00*

*Akadémiai Kiadó, Budapest, Hungary*
*Springer, Dordrecht, The Netherlands*

sity of microorganisms and thus diminishing the future soil richness. Therefore, it can be assumed that the living phase is finally responsible for the productive potential of soil [4].

- The evolution of the living soil phase, and the microbial activity in soil can be quantified by microcalorimetry [2, 5–10].
- As the microbial activity is strongly influenced by environmental features, mainly humidity and temperature, soil quality depends on physical, chemical and biological features and also on the environmental conditions. The investigations must be complemented by a deep analysis of the various mentioned soil factors of those environmental properties with a strong influence on the microbial activity in soil [2, 11–13]. The results calculated from different tests along the year must be analysed together in order to obtain a real knowledge of the global soil behaviour.

For a better understanding of this work, it has to be taken into account that the experimental approach was developed after 16 years of intensive scientific investigations.

## Experimental

Design and development of the experimental procedure for analyzing soils begins in 1993 [14] when the Research Group TERBIPROMAT studied the degradation of glucose by soil microorganisms using a microcalorimetric technique. In those experiments, soil samples were obtained from a forest in Pedroso (Santiago de Compostela, Spain) through a basic sampling procedure which was improved during the last years. Some properties of soil, such as water holding capacity, percentages of carbon and nitrogen, C-to-N ratio, pH and water content were also determined. All experiments were carried out at 35°C using a TAM 2277 of Thermometric AB (Sweden). Measurements were carried out in hermetically sealed 5 mL stainless steel ampoules. Soil samples of 1 g size at water-holding capacity were treated with 1.25 mg of glucose. Experiments were repeated four times. The reference ampoule was filled with 1 mL of distilled water. Table 1 presents the amounts of glucose degraded by soil microbes, $\Delta S=S_0-S_t$, between time '0' and time '$t$', determined by chemical analysis. The most important contributions of this study were:

- The demonstration that the value of the microbial growth rate constant μ can reasonably be regarded as the specific degradation rate of glucose and that it should be used as an index term to express how fast organic material is decomposed by microbial action.

**Table 1** Amount of glucose, ($S_0$–$S_t$), consumed by the soil microorganisms, determined by chemical analysis at different times [14]

| Time/h | $Q_T$/J g$^{-1}$ | ($S_0$–$S_t$)·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 |

$S_0$ – the initial amount of glucose in glucose mg$^{-1}$,
$S_T$ – the amount of glucose at time $t$ (after peak time) in glucose mg$^{-1}$

- The obtained relationship between the heat evolution of soil samples and the microbial activity permits the quantification of kinetic parameters for the microbial activity.
- The drop of the power–time ($P$–$t$) curve to the baseline is due to the exhaustion of glucose and not to the 'supposed' excess of $CO_2$ or a lack of oxygen (?).
- The confirmation of the importance of the temperature in the process of glucose degradation by microorganisms present in soils.

Once these conclusions were well established, the influence of different temperatures on the microbial growth was studied by Prof. Núñez concluding that the best temperature for Galician soils is about 25°C. Nowadays, different experiments are carried out at 20°C because this is the mean temperature in Galician soils along the year.

Following the experimental procedure of the previous paper [14], studies were performed to determine the effect of storage of soil at 4°C on the microbial activity determined by microcalorimetry [15]. The main conclusion was that important changes of the metabolic activity of microorganisms appeared when stored at 4°C. They are seen in the decrease of both, the total heat evolution, $Q_T$, and the growth rate constant μ. Since this moment it was assumed that storage of soil at 4°C for 3 months is an appropriate time to be sure that all microorganisms begin to grow at the same time and under the same conditions when they are brought back to the correct experimental temperature. Table 2 shows the values of the Peak-time ($P_t$), Peak-height ($P_h$), $Q_T$ and μ obtained from $P$–$t$ curves in this study. In the last time, this 'storage effect' is checked again because an anomalous behaviour was observed in different soils, especially in agricultural ones.

**Table 2** Characteristic microcalorimetric values calculated from *P–t* curves. These data were obtained from fresh and stored soils during 3 and 6 months [15]

| Sample | Peak–time/h | Peak–height/W $g^{-1}$ | $Q_T$/J $g^{-1}$ | $\mu/h^{-1}$ |
|---|---|---|---|---|
| Fresh soil | 14.75±0.29 | 8.54±0.38 | 15.17±0.37 | 0.155±0.002 |
| 3 months | 18.67±0.30 | 7.66±0.38 | 11.19±0.42 | 0.100±0.006 |
| 6 months | 33.17±1.21 | 7.28±0.16 | 9.05±0.56 | 0.019±0.003 |

$Q_T$ (J $g^{-1}$) – the total heat evolved during the processes, peak time – the time to reach the maximum of the peak, peak height (W $g^{-1}$) – the total heat evolved up to the maximum of the power–time curve, $\mu$ ($h^{-1}$) – the microbial growth rate constant. The final result is 14.75±0.29 h, where 14.75 h is the mean value calculated from 4 microcalorimetric experiments, and ±0.29 h is the standard deviation

**Table 3** Values of $\mu$ at different temperatures [20]

| Temperature/K | $\mu/h^{-1}$ |
|---|---|
| 288.15 | 0.026±0.005 |
| 293.15 | 0.033±0.005 |
| 298.15 | 0.047±0.005 |
| 303.15 | 0.057±0.004 |

**Table 4** Values of $\Delta G^{\neq}$ at different temperatures [20]

| Temperature/K | $\Delta G^{\neq}$/kJ $mol^{-1}$ |
|---|---|
| 288.15 | 98.85±0.44 |
| 293.15 | 100.03±0.22 |
| 298.15 | 100.92±0.29 |
| 303.15 | 102.40±0.17 |

## Results and discussion

Two years before, the Research Group TERBIPROMAT had studied different processes to optimize the microcalorimetric technique [16–19] in order to use it in various research fields.

The effect of different temperatures on the microbial degradation of glucose in soils was studied in 1997 [20]. An increase in $\mu$ with temperature was observed, that corresponded to the usual $Q_{10}$ rule with a rate increase of 2 to 3 for a temperature elevation of 10°C. Table 3 shows the values of the microbial growth rate constant at different temperatures. It is well known, that the temperature influence on these processes is determined by the activation energy *E*. This energy can be calculated from the Arrhenius equation:

$$k = Ae^{\frac{-E_a}{RT}}$$

where *k* is the rate constant, *A* the so-called pre-exponential factor, *R* the gas constant and *T* the absolute temperature.

Assuming that the pattern of bacterial growth is similar to the transition-state theory of chemical processes, it can be considered that:

$$\log\mu = \frac{-E_A}{2.303R}\frac{1}{T} + C$$

where *C* is a constant.

Then, Gibbs free energy changes associated with this processes can be calculated using the following equation:

$$\Delta G^{\neq} = RT\ln\frac{kT}{\mu h} = RT\left(\ln\frac{kT}{h} - \ln\mu\right)$$

Values of $\Delta G^{\neq}$ at different temperatures are compiled in Table 4.

The main conclusion of this work was that studies of temperature effects on ecological systems are important, because the microbial activity is very sensitive to temperature changes.

An improvement of the experimental protocol was done in 1999 [21] when Prof. Núñez determined the influence of glucose concentration on microbial activity in soil. The main conclusion of this research was that the optimum concentration of glucose was 1.25 mg glucose $g^{-1}$ soil. The values of the different parameters obtained in this study in the exponential phase are listed in Table 5. Recent studies of agricultural soils have shown that 'rich' soils of agricultural origin need higher amounts of glucose to develop a sufficient growth. More studies are performed by TERBIPROMAT to find the optimum concentration of glucose for each kind of soil. For example: a concentration of 2.50 mg glucose $g^{-1}$ soil is studied in agricultural soils with promising results.

Once the essential parameters for microcalorimetric measurements were obtained, the next step was to improve the sampling method in order to gain reproducible and representative results. At the same time, the influence of some physico-chemical parameters on the microbial growth in soils with different agricultural applications was analysed. That was the reason why the influence of different environmental parameters such as ambience and soil temperatures, moisture content (sample and residual), pH of water, and C/N ratio were introduced in 2002 [13]. Microcalorimetric techniques and environmental, physico-chemical and biological parameters were studied together for the first time [13]. It was concluded that cultivated soils showed a greater micro-

**Table 5** Values of main parameters used in this study with different concentrations of glucose. The microcalorimetric values correspond to the exponential phase obtained from *P*–*t* curves. $S_0$ is the initial amount of glucose (in glucose $mg^{-1}$), $S_T$ the amount of glucose at time *t* (after peak time) in glucose $mg^{-1}$, $N_0$ is the initial amount of biomass, $N_T$ the amount of viable mass at time *t* [21]

| | $S_0$ in mg glucose $g^{-1}$ soil | | | |
|---|---|---|---|---|
| | 1.25 | 5 | 20 | 50 |
| $Q_T$/J $g^{-1}$ | 3.58±0.40 | 4.95±0.20 | 11.53±0.21 | 16.49±0.52 |
| Peak-time/h | 13.60±0.46 | 17.10±0.72 | 26.25±0.29 | 45.83±1.42 |
| Peak-height/J $g^{-1}$ | 6.56±0.51 | 9.17±0.25 | 15.95±0.32 | 41.30±0.84 |
| $N_0/g^{-1}$ soil | $2.30 \cdot 10^8$ | $2.30 \cdot 10^8$ | $2.30 \cdot 10^8$ | $2.30 \cdot 10^8$ |
| $N_t/g^{-1}$ soil | $5.30 \cdot 10^8$ | $7.11 \cdot 10^8$ | $14.10 \cdot 10^8$ | $22.75 \cdot 10^8$ |
| $S_t$/mg glucose $g^{-1}$ soil | 0.00 | 0.02 (0.36%) | 0.13 (0.71%) | 0.19(0.38%) |
| $\mu/h^{-1}$ | 0.062±0.003 | 0.066±0.001 | 0.069±0.002 | 0.050±0.002 |

**Table 6** Main characteristic microcalorimetric parameters for different forest formations in autumn: 1 – *Eucalyptus globulus* Labill (eucalyptus), 2 – *Pinus pinaster* Aiton (maritime pine), 3 – *Pinus sylvestris* L. (wild pine), and 4 – Atlantic-humic climate forest (oaks, maple trees, alders, birchs and willows) [2, 23, 24]

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| $P_t$/h | 175.75±1.77 (1.01%) | 19.62±0.32 (1.64%) | 10.75±2.25 (0.65%) | 20.27±0.24 (1.18%) |
| $P_h/\mu W$ | 21.09±0.92 (4.35%) | 144.03±1.10 (0.76%) | 71.65±0.52 (0.73%) | 0.91±0.01 (1.31%) |
| $Q_t$/J $g^{-1}$ | 6.32±0.21 (3.26%) | 885.02±1.88 (0.21%) | 345.05±2.25 (0.65%) | 1.73±0.05 (2.74%) |
| $\mu/h^{-1}$ | 0.00120±0.00001 (1.09%) | 0.0936±0.0020 (2.15%) | 0.0106±0.0004 (3.57%) | 0.0309±0.0010 (3.38%) |

Peak time, $t_{max}$ $P_t$ (h) – is the time to reach the maximum of the peak, peak height, $P_h$ (μW) – is the power at the maximum of the peak, $Q_t$ (J $g^{-1}$) – is the total heat evolved up to the maximum of the power–time curve and μ ($h^{-1}$) – is the microbial growth rate constant

bial activity opposite to the no cultivated soils. The cultivated soils kept their properties nearly constant in values considered as optimal to assure adequate soil behaviour, both from physico-chemical and biological points of view. Because of this, a rational exploitation of soils allows a sustainable and stable microbial population, thus indicating the maintenance of soil productivity.

Following this research line, the investigations were focussed on different forest species in particular studies. In 2005 and 2006, new papers appeared analysing the influence of agricultural exploitation on the control of the productive capacity of soils [2, 22–24]. In particular, this study analysed the influence of two forest species, a pine and a fast growing species such as eucalyptus [2, 23, 24], on a typical soil of the zone, a humic Cambisol, which is usually covered by an Atlantic-humid climate forest. Both papers were pioneering and examples of combined studies, where thermal, environmental, physico-chemical, and biological parameters had the same importance [23, 24]. The most important characteristic microcalorimetric parameters are depicted in Table 6. Moreover, an essential improvement of the sampling procedure was described [23, 24]. Analyses of the power-time curves lead to a deeper understanding of microbial activity in similar soils [2, 14–15, 20–24]. Today, the experimental procedure is improved by the determination and classification of different microbial communities found in soil. The main objective of these improvements is to introduce new techniques to assay the obtained results, to analyse the $CO_2$ generated in soils, and other questions.

## Conclusions

The data presented here, are a summary of the conclusions obtained in the eight most important of our scientific papers. This study demonstrated definitely that the microbial activity, and of course soil behaviour, greatly depend on the environmental conditions and on the forest cover. The combined study of calorimetric, physical, chemical, biological and environmental features provides rapid and suitable information about microbial activities and soils' health state. This experimental procedure proposed and developed by Prof. Núñez and TERBIPROMAT has been success-

fully checked during more than 16 years using different kinds of soils, both in their natural status and in use, situated in various zones of Galicia. In our opinion, this experimental procedure could be used to study any type of soil.

A further important aim was to probe the advantage of microcalorimetry over the other techniques, based on the continuous monitoring of soil microbial activity. Microcalorimetry provides 'real information' leading to the determination of the productivity potential. Results obtained after a comparative study of similar soils subjected to different human activities could be the basis for campaigns designed to recover degraded soils or to avoid soil degradation. When this study is finished, it should be helpful to create a data basis to quantify and control soil potentiality for a rational and sustainable exploitation of the resources.

## Acknowledgements

The authors wish to thank the Vicerrectorado de Investigación, University of Santiago (Spain). An important part of this research was sponsored by Xunta de Galicia through the fund projects PGIDIT04REM206001PR and PGIDIT04PXIB20601PR.

We apologize for any omission. We know that this so special paper was possible thanks to a lot of friends and colleagues of Prof. Núñez, but it would be impossible to thank all of them here. We thank from the bottom of our hearts all those people that helped us every time we needed it and whom we can not name here: scientific collaborators, colleagues, friends, editors of the different scientific publications, referees, and many others.

## References

1 G. P. Sparling, Soil Biology Biochem., 13 (1981) 93.
2 L. Núñez-Regueira, J. A. Rodríguez-Añón, J. Proupín-Castiñeiras, O. Núñez-Fernández and M. Villanueva-López, Soil Biol. Biochem., 38 (2006) 115.
3 B. Chardin, P. Gallice, J. C. Sari and M. Bruschi, J. Therm. Anal. Cal., 70 (2002) 475.
4 R. Y. Stanier, E. A. Adelberg and J. L. Ingraham, Microbiología, Reverté-Repla S. A., Barcelona 1985, p. 262.
5 I. Wadsö, A. E. Beezer, Biological Microcalorimetry, Academic Press, London 1980, p. 247.
6 K. Ljunghlom, B. Norén, R. Sköld and I. Wadsö, Oikos, 33 (1979) 24.
7 I. Lamprecht, Biological Microcalorimetry, Academic Press, London 1980, p. 43.
8 H. Yamano and K. Takahashi, Agric. Biol. Chem., 47 (1983) 1493.
9 J. P. Belaich, Biological Microcalorimetry Academic Press, New York 1980, p. 1.
10 W. W. Forrest, J. R. Norris and D. W. Ribbons (Eds), Methods in Microbiology, Academic Press, London 1972, p. 285.
11 L. D. Baver, W. H. Gardner and W. R. Gardner, Soil Physics, John Wiley & Sons Inc., New York 1991, p. 78.
12 J. K. Mitchell, Fundamentals of Soil Behaviour, John Wiley & Sons Inc., New York 1993.
13 L. Núñez-Regueira, O. Núñez-Fernández, J. A. Rodríguez Añón and J. Proupín Castiñeiras, Thermochim. Acta, 394 (2002) 123.
14 L. Núñez, N. Barros and I. Barja, Thermochim. Acta, 237 (1994) 73.
15 L. Núñez, N. Barros and I. Barja, J. Thermal Anal., 41 (1994) 1379.
16 L. Núñez Regueira, I. Gómez-Orellana, N. Barros Pena, R. Chamy and J. M. Lema, Thermochim. Acta, 172 (1990) 163.
17 A. F. Danil de Namor, D. A. Pacheco Tanaka, L. Núñez Regueira and I. Gómez Orellana, J. Chem. Soc. Faraday Trans., 88 (1992) 1665.
18 I. Gómez Orellana, N. Barros, I. Barja and L. Núñez Regueira, Thermochim. Acta, 219 (1993) 135.
19 R. Chamy, L. Núñez-Regueira, I. Gómez, M. J. Núñez and J. M. Lema, Biotechnol. Techn., 9 (1995) 231.
20 M. I. Barja, J. Proupín and L. Núñez, Thermochim. Acta, 303 (1997) 155.
21 I. Barja and L. Núñez, Soil Biol. Biochem., 31 (1999) 441.
22 L. Núñez-Regueira, J. A. Rodríguez-Añón, J. Proupín-Castiñeiras and O. Núñez-Fernández, J. Therm. Anal. Cal., 80 (2005) 35.
23 L. Núñez-Regueira, J. A. Rodríguez-Añón, J. Proupín-Castiñeiras, M. Villanueva-López and O. Núñez-Fernández, J. Therm. Anal. Cal., 84 (2006) 7.
24 L. Núñez-Regueira, J. Proupín-Castiñeiras, J. A. Rodríguez-Añón, M. Villanueva-López and O. Núñez-Fernández, J. Therm. Anal. Cal., 85 (2006) 271.

DOI: 10.1007/s10973-006-7844-3