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# The influence of moisture on microbial activity of soils

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**Abstract**

A series of microcalorimetry experiments were performed to follow the effect of moisture on soil microbial activity. Activity was stimulated by adding a solution of 6.0 mg of glucose and 6.0 mg of ammonium sulfate in 0.80 $cm^3$ of distilled water to 1.50 g of red Latosol soil. This solution represents the moisture used in the experimental system (MAES) which directly affects the microbial activity. MAES values vary from 9.1 to 50.0% in selected reports where the humidity was not taken into account in calorimetric determinations. In this study, the thermal power caused by microbial activity was followed in samples of red Latosol with MAES values of 34.78%. In soil with absolute humidity values varying between 36.74 to 37.74%, the total heat reached a maximum value, 15.92 J $g^{-1}$. A drastic decrease above absolute moisture values of 41.82% was observed. The methodology with 34.78% MAES values showed a better microbial activity on soil than lower values of humidity. The proposed methodology establishes a defined MAES value of 34.78% to optimize the calorimetric results obtained from air-dried soil experiments. 

*Keywords:* Soil; Microcalorimetry; Metabolism; Microorganisms

## 1. Introduction

Soil is the most important region of the geosphere and from it a great variety of foods, minerals and fuels are obtained [1–3]. Soil is an open system where several physical, chemical and biological reactions may occur in the interior and on the surface. Both matter and energy can be exchanged with the surroundings [4].

Metabolic activities of living organisms result in a final exothermic effect in nature. The pioneer measurement involving metabolic heat generated by a guinea pig in an adiabatic calorimeter was made by Lavoisier and Laplace in 1780. Such measurements have influenced many researchers in monitoring the thermal power released from different biological systems. Bioprocesses have been successfully correlated to thermal effects and the quantity of thermal power with the bioactivity of systems and processes [5]. The development of microcalorimetry was fundamental to study of microbial and the cellular systems [6–8]. The activities of a population of microorganisms generate a thermal power which can be related to a corresponding enthalpy determined through calorimetric measurements [9].

Calorimetric techniques are very suitable for this kind of investigation because a continuous signal recording of a life process can be followed for long periods of time without disturbing the development of the system. However, for a given microorganism

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population, the degradation of nutrients depends on several features, such as nutritional conditions, pH, temperature, moisture, oxygen levels, etc. The importance of soil moisture in regulating microbial activity is well known [10,11]. It influences a number of physical and chemical properties of soil, such as redox potential, pH, oxygen and carbon dioxide levels [10,11], which can also influence the microbial population and overall activity. A series of selected reports on microbiologic activity in soil by use of microcalorimetry presents very different MAES values. The following MAES values were found: 9.09% [11–13], 14.29% [14], 16.67% [15,16], 33.33% [17], 34.78% [9,18,19], 50.0% [5,20–22]. These differences hinder comparisons of microbiologic activity. Standardization of moisture level would permit comparison of results.

## 2. Experimental

### 2.1. Reagents

All chemicals used, such as glucose (Hoescht), ammonium sulfate (Baker) and potassium chloride (Carlo Erba) were reagent grade.

### 2.2. Soil samples

Red Latosol soil, which covers around 15% of the State of São Paulo, was collected on the campus of the Universidade Estadual de Campinas. Samples were collected to a depth of 5 to 10 cm, after removal of the top surface layer [9], and were homogenized by sieving to less than 2 mm to separate roots and large particles [23]. The soil was stored in polyethylene bags at $293 \pm 5$ K for at least three months before being used in the calorimetric experiments.

For organic matter determination, triplicate samples of air-dried soil were placed in a muffle at a temperature of 823 K for 24 h to follow the decrease in mass, as recommended [24,25]. Under these conditions organic matter is combusted leaving only the inorganic portion of the soil.

Carbon, nitrogen, hydrogen and sulfur contents in soil were determined in triplicate by elemental analysis by using an Elemental Analyzer Fisons Instruments CHNS-O model 1110.

Measurements of pH were obtained in triplicate by means of a pHmeter Digimed DMPH-2. In such determinations 2.0 g of soil sample were suspended in 5.0 $cm^3$ of a strong electrolyte such as 1.0 mol $dm^{-3}$ calcium chloride in a proportion of 1 : 2.5 for soil : solution (*m*/*v*) [24–27].

### 2.3. Moisture of soil

The moisture of four distinct aliquots of soil, named RLSX ($X = 1$ to 4) was determined in triplicate. The first one represents the original soil. Aliquots 2, 3 and 4 were dried for 5, 10, and 15 days, respectively. The degree of moisture was obtained by weighing 2.0 g of dry soil in a crucible, previously dried in an oven at 383 K. The final moisture content was determined from the mass lost in the oven at same temperature for 24 h [24,25,27]. Under these conditions a constant mass was obtained.

### 2.4. Microcalorimetry

A heat-conduction microcalorimeter (LKB 2277) was used for all measurements. This instrument, called a Thermal Activity Monitor, 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 μW, baseline noise < 0.2 μW, working temperature 293–353 K, thermal stability better than $2.0 \times 10^{-4}$ K over a period of several days at the temperature of measurements. The thermal power was obtained using 5.0 $cm^3$ stainless steel ampoules. These ampoules were hermetically closed by Teflon sealing discs to avoid evaporation inside the apparatus. Experiments were carried out at $298.15 \pm 0.02$ K. All determinations of the thermal power were performed in triplicate in ampoules containing 1.50 g of soil and 0.80 $cm^3$ of solution containing 6.0 mg of glucose plus 6.0 mg of ammonium sulfate [9,18,19]. Under these conditions moisture applied on experimental system (MAES) values were calculated as being 34.78%. The thermal power associated with nutrient degradation was recorded as a function of time. The final total heat value was calculated from the integrated area of the power time curves.

## 3. Results and discussion

Physical and chemical characteristics such as pH, organic matter, carbon, nitrogen, hydrogen and sulfur contents are important because microbial behaviour depends on these properties. This soil contained $5.50 \pm 0.22\%$ of organic matter, $5.22 \pm 0.11\%$ of carbon; $0.92 \pm 0.04\%$ of nitrogen and $2.73 \pm 0.14\%$ of hydrogen, and has pH of $5.84 \pm 0.06$.

The intrinsic moisture of a given soil is established from its water content, according to the relationship:

$$\mathrm{IM} = \frac{(m - m_s)}{m} \times 100,$$

where IM is intrinsic moisture, and $m$ and $m_s$ are masses of humid and dry soils, respectively. In routine calorimetric investigations on microbial activity in soils, it is necessary to add a nutrient-carrying solution which increases the water content. The second relative moisture is defined by

$$\mathrm{MAES} = \frac{(m_{sol} - m_s)}{m_{sol}} \times 100$$

where $m_s$ is the mass of dry soil, and $m_{sol}$ the sum of mass of dry soil and mass of solution of nutrients.

Thus, the real moisture (AM) is given by the sum of the intrinsic moisture (IM) content of the soil plus the MAES values, calculated through the expression $\mathrm{AM} = \mathrm{IM} + \mathrm{MAES}$.

Table 1 shows how different extents of soil drying affect moisture before and after soil wetting. As expected, the increase in time of air drying caused a decrease in the percentage of moisture per gram of the samples of soils with fixed value of MAES, and consequently, a decrease of AM values.

The present results with 1.50 g of soil with a MAES value of 34.78%, but varying AM values as indicated in Table 1 are illustrated in Fig. 1.

Table 1
Intrinsic moisture, IM (%), and absolute moisture, AM (%), determined in samples of red Latosol soil air dried for various times

| Sample | Time (d) | IM (%) | AM (%) |
|---|---|---|---|
| RLS1 | Original | $18.22 \pm 0.38$ | $53.00 \pm 0.38$ |
| RLS2 | 5 | $7.04 \pm 0.11$ | $41.82 \pm 0.11$ |
| RLS3 | 10 | $2.96 \pm 0.04$ | $37.74 \pm 0.04$ |
| RLS4 | 15 | $1.96 \pm 0.11$ | $36.74 \pm 0.11$ |

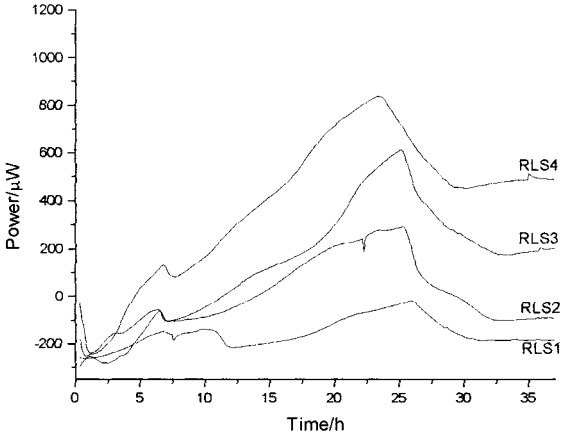


Fig. 1. Power–time curves of the microbial activity of soils RLSX ($X = 1$ to 4), at different percentages of absolute moisture: 53.00, 41.82, 37.74 and 36.74, respectively.

The profiles of the power-time curves shown in Fig. 1 are very similar. All curves gave the same peak time, around 25 h.

Thermal power values are different at the various levels of moisture. These results show that humidity directly influences the microbial activity of soil. However, it is also important to consider the total heat per gram of soil, Qm. Variations in the total heat liberated were observed, Table 2.

These results show that Qm values were highest at for samples with AM values varying between 36.74 and 37.74%, reaching to 15.9 J $g^{-1}$. Slightly lower results for Qm are observed between 36.74 and 41.82% in AM. For samples with AM values above 41.82% the total heat decreases to 6.66 J $g^{-1}$.

From the values listed in Table 2, it is easy to conclude that the highest value for total heat was 15.92 J $g^{-1}$ in 37.74% of AM value. This result is in accordance with those obtained by Barros et al.

Table 2
Calorimetric results of microbial activity for samples RLSX ($X = 1$ to 4) of red Latosol, to give the total heat Qt (J), total heat obtained per 1 g of soil Qm (J $g^{-1}$) and peak time manifested at maximum PT (h)

| Sample | −Qt (J) | −Qm (J $g^{-1}$) | PT (h) |
|---|---|---|---|
| RLS1 | $8.17 \pm 0.64$ | $6.66 \pm 0.68$ | $26.09 \pm 0.98$ |
| RLS2 | $20.41 \pm 0.82$ | $14.64 \pm 0.83$ | $25.67 \pm 1.02$ |
| RLS3 | $23.18 \pm 0.24$ | $15.92 \pm 0.25$ | $25.32 \pm 0.52$ |
| RLS4 | $23.37 \pm 0.54$ | $15.89 \pm 0.66$ | $23.79 \pm 0.75$ |

[11], who reported a MAES of 9.09% which gave the highest Qm value at 38.1% in AM value, with an observed decrease of the activity above this value.

## 4. Conclusion

The present results confirm that moisture has an effect on microbial activity of soils. The microbial activity has a maximum value between 36.74 and 37.74% in AM values. Above 41.82% of AM, the total heat decreases. Thus, the moisture applied to any experimental system is a decisive factor to optimize experiments in the microcalorimetry of soils. The presentation of moisture data in studies of microbial activity of soil by microcalorimetry must be done in terms of absolute moisture, because only AM represents the real moisture of the system.

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