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Microcalorimetric study of glucose degradation in some Brazilian soils

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Abstract

Latosol soils, red, dark red, red-yellow, and red-yellow treated with vinasse, were studied by microcalorimetry. Microbial activity was stimulated by the addition of identical masses of glucose with ammonium sulphate, varying from 3.0 to 12.0 mg per 1.5 g of soil (glucose being a limiting energy source) under controlled humidity at 298 and 306 K. After dosing all the soil samples (1.5 g) with 6.0 mg of each nutrient and 0.8 cm^3 of water, the power-time curves were recorded. These curves showed that red soil gave the largest power output (240 μW) at a peak time of 35.2 h (the point of maximum amplitude of the experimental curve). Yellow soils, however, did not show any response to addition of nutrients. The power–time curves are all similar in shape, with the thermic effect and the peak time for both temperatures being directly dependent on the amount of nutrient. The peak time decreases with increasing temperature; values of 29.2–43.8 h at 298 K and 17.3–23.8 h at 306 K, were obtained.

INTRODUCTION

Soil can be considered as a multicomponent, open biochemical system, conglomerated by solids, liquids and gases [1]. Because it is an open system, several physical, chemical and biological reactions may occur inside and on the surface, both matter and energy also being exchanged with the surroundings. Each system disturbance is accompanied by either heat evolution or heat adsorption [2]. The degree of intensity of this disturbance is directly correlated with the composition of the system. Thus, in the study of an individual soil system, the matter, energy and information concerning the system pertain to the organisms and populations which constitute the life development and environment of this open system. These activities generate a flow of heat caused by an increase or decrease in the energy

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sources, resulting from metabolic changes which can be related to the enthalpy change determined by caloric measurements [3–5].

Caloric techniques are very suitable for this kind of investigation [6–10] because a continuous recording of the signal of a life process can be followed for longer times without any disturbance of the system, unlike the situation with sampling procedures. Hence, calorimetry permits in situ determination of a given microbial activity. In this particular case, microcalorimetry was used successfully to investigate microbial activities in soils, not only to characterize the activity, but also to determine the inhibitor effect of foreign substances on the microbial degradation of a carbon source in soil [11, 12]. For a given microorganism population, the degradation depends on the nature of the carbon source added to the soil, as has been demonstrated for different sugars [8].

In the present investigation, samples of natural soils of different origins were studied by varying the amount of nutrient and temperature. The microbial degradation of glucose was monitored calorimetrically as a typical power–time curve in order to characterize the system at different temperatures and to correlate the heat produced during the degradation of glucose.

EXPERIMENTAL

Reagents

All chemicals used were reagent grade. Glucose (Hoescht), ammonium sulphate (Baker), sodium nitrate (Ecibra), hydrochloric acid (Merck), sodium hydroxide (Quimis), nitric acid (Merck), calcium chloride (Carlo Erba), magnesium sulphate heptahydrate (Ecibra), potassium hydrogenphosphate trihydrate (Merck), potassium dichromate (Quimis), iron ammonium sulphate hexahydrate (Vetec), calcium acetate (Ecibra), potassium chloride (Carlo Erba), diphenylamine (Merck), tetracycline (Klinger), chloramphenicol (Carlo Erba) and cellulose (Rhodia) were used.

Soil samples

The main objective in sampling was to have representative Brazilian soils, bearing in mind the extent of this territory and the range of useful agriculture soils which are mainly located in the region of the State of São Paulo. Therefore, four different soils were collected: (i) rich red soil, called red Latosol, which covers around 15% of the state, was obtained directly from the campus of the University; (ii) dark red Latosol soil, originating

from a sugar cane plantation field; (iii) poor sandy soil, characterized by bush vegetation under cerrado (savanna), called red-yellow Latosol; and (iv) the red-yellow Latosol treated with vinasse (the residue from the industrial production of ethanol from sugar cane by fermentation and subsequent distillation) [13], which is a common procedure to increase the pH and to enrich the soil with organic matter.

The samples were collected from a depth of 5–10 cm, after removal of the top surface layer. The soil was dried in air for one week and sieved (0.59 mm) to separate root fragments and large particles. This soil was stored in polyethylene bags at 293 ± 5 K for at least three months before being used for the calorimetric experiments [14–16].

Characterization was carried out by routine methods. The percentage of water per gram of dry soil was determined by drying the sample to constant mass [15]. The percentage of organic matter per gram of dry soil was obtained by titrating the soil in an acid medium, with the end point being followed by a redox reaction [14, 17]. The pH measurements were obtained in a strong electrolyte such as calcium chloride (1.0 mol dm^{-3}) in a proportion of 1:2.5 for solution:soil. The total acidity (H^+ plus Al^{3+}) was determined by percolating 5.0 g of dry fine soil in air (DFSA) with 0.10 dm^3 of calcium acetate (2.0 mol dm^{-3}) at pH 7.0. The eluate was titrated with sodium hydroxide (0.05 mol dm^{-3}). The cation-exchange capacity was obtained from the sum of the extractable bases and the total acidity. The base contents were determined by extracting the percolated fraction of 10.0 g of the same soil (DFSA) with 0.10 dm^3 of nitric acid (0.05 mol dm^{-3}). Calcium and magnesium were determined in the same extracted fraction using a Perkin-Elmer model 5000 atomic absorption spectrometer with an automatic absorption control, and potassium was determined using a Micronal model B 262 flame photometer.

Sterilized samples

Red Latosol soil samples were treated with hydrochloric acid (12.0, 5.0 and 1.0 mol dm^{-3}). Each sample was kept in its respective solution for 4 h, after which the solution was decanted and filtered. The soil was washed several times with distilled water, to reach pH 7.0 [2]. After drying in air, the colour was grey.

Calorimetry

The LKB 2277 heat-flow microcalorimeter used for all measurements has been previously described in detail [18–21]. 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, detection sensitivity 0.4 V W^{-1}, working temperature 293–353 K, thermal stability better than 2.0×10^{-4} K over a period of several days at the temperature of measurement. The thermic effect was obtained using 3.0-cm^3 glass ampoules, sealed with aluminium caps with an integral seal. A small hole was made in the rubber of the cap, and then covered with polyethylene before sealing [6]. One ampoule, with 1.50 g of soil plus 0.80 cm^3 of water, served as a reference; the other ampule, in addition to sample, contained the desired reagent as substrate. Both ampoules were exposed to the ambient environment of the thermostated cylinder. Thermal equilibrium was attained after 3 h. Thus, the calorimeter signal was recorded as a power–time curve. The thermic effects produced by the soil samples were sufficiently slow in this process to allow the power signal to be considered as being proportional to the effect caused by the samples in that time, because the calorimeter time constant is approx. 180 s.

The calorimetric measurements were performed at 298 and 306 K and a good baseline was obtained about 2–3 h (normally, 3 h) after the start of the experiment. In a general procedure, the experiments were performed by adding X mg of ammonium sulphate plus X mg of glucose, where $X = 3.0$, 6.0 or 12.0 mg. A blank experimental run containing glucose, ammonium sulphate and water showed no baseline deviation. It has been proposed that the behaviour of microorganisms in soil changes completely when water is removed [6]. In experiments carried out under this condition, no deviation of the baseline was detected; consequently, because water was used in all runs, the thermic effect for comparison was based on experiments with glucose as a limiting energy source at the established water content.

The thermic effect for a given experiment was calculated by comparing the integrated areas of the power–time curves, which correspond to the experiment, and that of the electrical calibration.

RESULTS AND DISCUSSION

The four selected soils have nearly the same water content. Differences in acidity, organic matter content, cation-exchange capacity and total acidity are summarized in Table 1. The red Latosols are comparable in organic matter content but differ in acidity. The soil with the highest pH represents the most fertile area chosen for cultivation in this state, and the dark red acid soil indicates some degradation of the soil as a result of the cultivation of sugar cane. The red-yellow Latosols also have similar values for organic matter content and acidity. Treating the soil with vinasse makes the savanna more suitable for cultivation, but its high aluminium content is toxic to plants; this is also reflected in the high cation-exchange capacity of the soil which is even greater without vinasse treatment.

TABLE 1

Water content H (%H_2O per g soil), organic matter content OM (% per g soil), pH (1.0 mol dm^{-3} $CaCl_2$), total acidity ($H^+ + Al^{3+}$) (mequiv. per 0.1 dm^3), cation exchange capacity CTC (mequiv. per 0.1 dm^3) and extractable bases SB (mequiv. per g soil)

Soil	H	OM	pH	($H^+ + Al^{3+}$)	CTC	SB
Red Latosol	1.3	3.3	5.2	3.8	9.2	5.4
Dark red Latosol	1.2	3.0	4.0	9.9	10.6	0.7
Red-yellow Latosol	1.7	4.6	3.5	15.0	16.4	1.4
Red-yellow Latosol treated with vinasse	1.6	4.8	3.6	12.2	12.9	0.7

The four soils showed remarkable differences when each was treated with 6.0 mg of glucose and 6.0 mg of ammonium sulphate at 298 K. A clear demonstration of this behaviour is shown qualitatively in Fig. 1. In this study, red Latosol soil presented the largest signal (240 μW) at a peak time of 35.2 h, which contrasted with the results for dark red Latosol: 180 μW and 84.0 h. The other two soils showed no response to the addition of nutrients over an experimental period of more than one hundred hours. Because of these preliminary results, the red Latosol soil was selected as a system for further study, because the microorganism population was correlated to the area of the power–time curve and to its maximum amplitude [11].

A control of the degradation of glucose for the twenty months of experiments of this work showed that the maximum amplitude of the curve increased smoothly with time, with a tendency to remain constant at the end of the period. In a normal assay, the system considered is formed by a sample containing 1.50 g of soil, 0.80 cm^3 of distilled water plus nutrients. For this control the nutrients were 6.0 mg of glucose plus 6.0 mg of ammonium sulphate. The thermic effect produced is compared to a

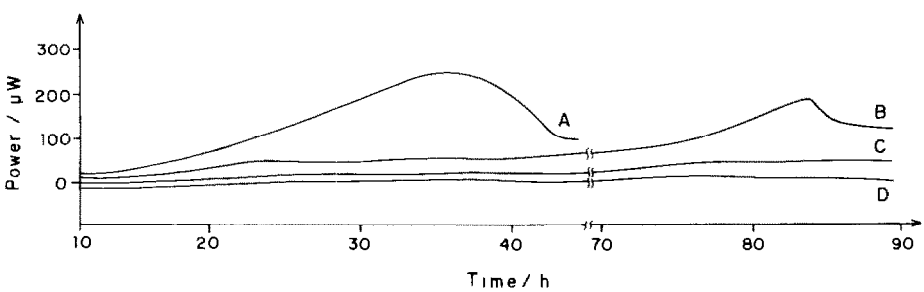

Fig. 1. Microbial degradation of 6.0 mg of glucose plus 6.0 mg of ammonium sulphate for different types of Latosol soils at 298 K: (A) red; (B) dark red; (C) red-yellow; and (D) red-yellow treated with vinasse.

reference sample with equivalent quantities of soil and water only. Thus, the observed signal results from biological or chemical processes. However, a contribution from chemical processes was rejected in a separate experiment.

The presence of microorganisms in the soil was assessed by sterilization with hydrochloric acid. The sterlilized soil had a very low thermic effect, in which the development of the normal exponential growth curve was not detected in 60 h after addition of nutrients. This is in agreement with other data obtained [6, 7] that have shown that if any non-biological reaction takes place in the soil, it is a minor contribution.

Glucose was the only added source of carbon used in the experiments reported here. Powdered cellulose with sodium nitrate, magnesium sulphate or potassium hydrogenphosphate, was also assayed, but because of its polymeric structure, it was a non-degradeable energy source for the components of the soils in this experimental period.

The degradation of glucose was studied on samples prepared under the same conditions; the power–time curves are essentially identical in shape, representing typical microbiological growth [2]. The area of the peak, is related to the degradation of glucose and reflects the amount of glucose used. The power output at the peak time and the time interval for the duration of the peak should be related to the population living in the soil sample. For samples having equivalent masses of glucose and ammonium sulphate, the shapes of the curves depend on the temperature. This behaviour is shown by the peak time, i.e. the time at which the experimental curve attained maximum amplitude. An increase in temperature shifts this maximum to a lower time with an increase in peak area, as a result of an increase in the thermic effect. Figure 2 illustrates an experiment with 6.0 mg of glucose plus 6.0 mg of ammonium sulphate at 298 and 306 K,

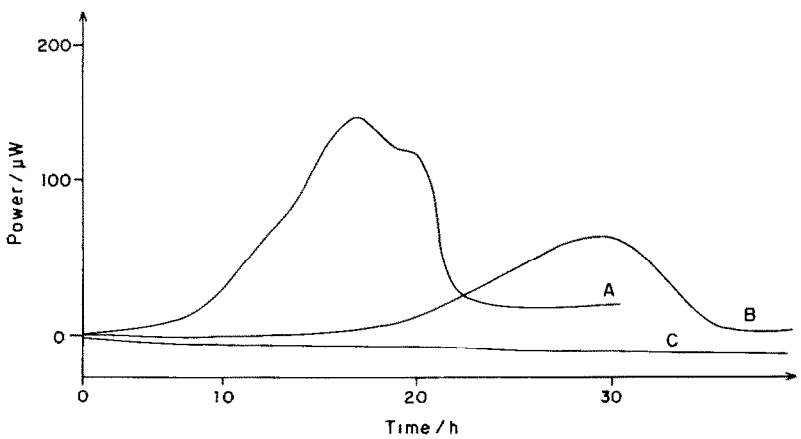

Fig. 2. Temperature effect on degradation of 6.0 mg of glucose plus 6.0 mg of ammonium sulphate for red Latosol soil at: (A) 306 K; (B) 298 K. Curve (C) is for nutrients without soil.

TABLE 2

Degradation of added glucose m in red Latosol soil at a distinct temperature T to give a peak time (t_p) in the interval time of duration of the peak Δt, providing the thermic effect Q_{obs} and the estimated glucose consumed m_{cons}

T/K	m/mg	t_p/h	$\Delta t \times 10^4$/s	$-Q_{obs}$/J	m_{cons}/mg
298	3.0	29.2 ± 1.3	8.4 ± 0.2	3.45 ± 0.09	0.225
298	6.0	35.2 ± 1.4	10.6 ± 0.2	6.75 ± 0.27	0.440
298	12.0	43.8 ± 1.8	15.5 ± 0.3	14.13 ± 0.57	0.922
306	3.0	17.3 ± 0.7	5.4 ± 0.1	4.26 ± 0.09	0.278
306	6.0	19.4 ± 0.8	7.2 ± 0.1	8.97 ± 0.36	0.585
306	12.0	23.8 ± 1.0	11.0 ± 0.2	19.35 ± 0.78	1.262

respectively. Table 2 summarizes the results obtained for both temperatures, using three different amounts of nutrient at each temperature. An increase in nutrient from 3.0 to 12.0 mg is followed by a similar increase both in peak time and in thermic effect, which is illustrated in Fig. 3 for 306 K. These thermic effect values are listed in Table 2; the values quoted are the mean value followed by twice the uncertainty of three individual determinations. The microorganisms residing in each sample partially consumed the nutrients at the peak time, causing a higher thermal effect for an increase in temperature, due to the enhancement of the microbial

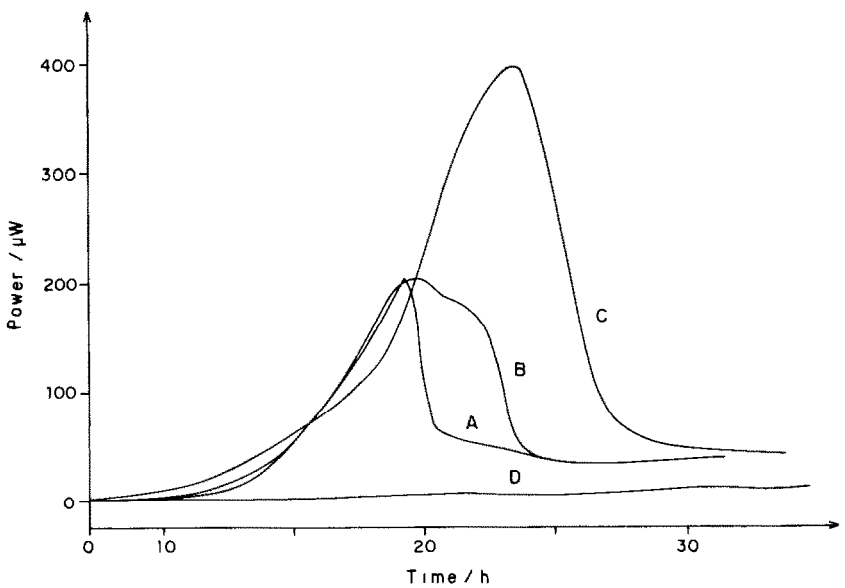

Fig. 3. Amount of glucose added to red Latosol soil at 306 K: (A) 3.0; (B) 6.0; (C) 12.0 mg; and (D) nutrients without soil.

growth in this soil in this case. The main reaction that occurs in the ampoules under aerobic conditions is

$$C_6H_{12}O_6(aq) + 6O_2(aq) \rightleftharpoons 6CO_2(aq) + 6H_2O(l)$$

From the enthalpy of this reaction in condensed phase, $\Delta_r H_m^{\ominus} = -2802\ kJ\ mol^{-1}$ [22], Hess' law was applied to correct the enthalpy of reaction under the experimental conditions. In this calculation, the standard enthalpies of solution of gaseous carbon dioxide, $-20.29\ kJ\ mol^{-1}$ [23], and oxygen, $-11.7\ kJ\ mol^{-1}$ [23], in water, and also of glucose, $+11.028\ kJ\ mol^{-1}$ [24], in water were considered, yielding $\Delta_r H = -2762\ kJ\ mol^{-1}$.

From the estimated amount of glucose consumed (m_{cons}), the microbial growth can be calculated by means of the equation

$$m_{cons} = Q_{obs.}(MM)/\Delta_r H$$

where MM is molar mass of glucose ($180.16\ g\ mol^{-1}$). In comparing the m and m_{cons} values listed in Table 2, it is clearly seen that about 10% of the glucose was consumed during the peak. This figure is in agreement with the experimental results, which showed that the tail of the peaks were always above the initial level of the baseline, suggesting that it takes a long time to consume all the glucose employed in each experiment.

The linear correlation between thermic effect or peak time with the amount of glucose added to the soil samples is illustrated in Figs. 4 and 5,

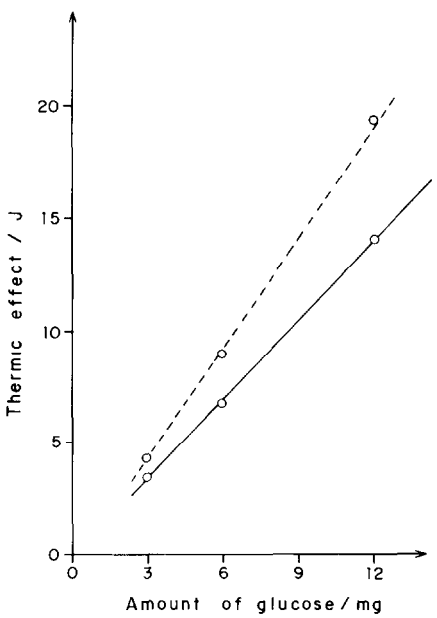

Fig. 4. Linear correlation between the thermic effect Q and the amount of glucose at 298 (——) and 306 K (– – –).

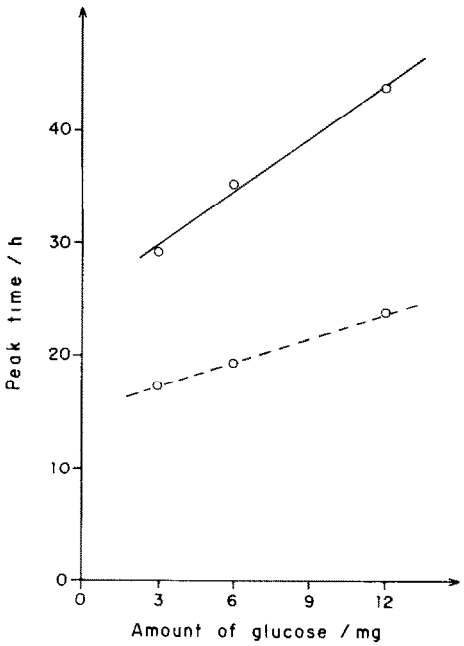

Fig. 5. Linear correlation between peak time and amount of glucose at 298 (——) and 306 K (– – –).

respectively. The shift of the peak time to lower values (Fig. 5) is accompanied by an increase in thermic effect (Fig. 4). This comparison demonstrates that the growth of microorganisms is favored at higher temperature. For example, adding 12.0 mg of glucose gave a peak time at 43.8 h for 298 K, but this changed to 23.8 h at 306 K, (see Table 2), and the corresponding thermic effect changed from 14.13 to 19.35 J.

The highest microorganism population in red Latosol can be evidenced under identical experimental conditions. For example, when this soil was fed with 6.0 mg of nutrients at 298 K, the peak time and the power output at this point were 35.2 h and 240 μW, respectively. These values contrasted with those obtained in identical conditions with the dark red Latosol from the sugar cane plantation, whose values were 84 h and 180 μW, respectively. Red-yellow Latosol and red-yellow Latosol containing vinasse had no response after nutrient application (Fig. 1).

To try to identify the nature of the microorganisms in the soil, 6.0 mg of an antibiotic, either tetracycline or chloramphenicol, were applied together with the nutrients at 298 K. In experiments with the first additive, a maximum value of power output of 100 μW was observed which is less than half the order of magnitude obtained from the experiment without antibiotic. In the experiment with chloramphenicol, the power–time curve remained at the baseline level, as observed before [25], suggesting that the antibiotics have distinct behaviours with respect to the inhibition of

microorganism growth. These values clearly show that chloramphenicol acts as a bactericide, with the capacity to suppress the metabolism of the microorganisms in the soil.

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