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The inhibitor effect of copper sulphate on microbial glucose degradation in red latosol soil

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Abstract

Microcalorimetry has been used in a series of experiments to study the inhibitory effect caused by copper sulphate on soil microbial activity. This activity was stimulated by addition of 6.0 mg of glucose and 6.0 mg of ammonium sulphate under a controlled humidity of 53% in a red Latosol soil sample of 1.50 g. Power-time curves were recorded for increasing amounts of the inhibitor, varying from zero to 6.19 mg. The curves showed a decrease of the maximum amplitude of the experimental curve, which shifted to longer times. Increasing masses of copper sulphate caused a decrease of the original thermal effect to reach a null value at 6.19 mg of inhibitor. The results relating the dependence of the maximum amplitude of the peak time with the considered pollutant mass, were fitted to a kinetic model in an attempt to establish the inhibitory effect of copper sulphate. In this conditions, the data were adjusted to a first power order model for the degradation of glucose. In the absence of inhibitor the consumption of glucose by the microorganisms is about 10% of the initial mass and decreases with the increase of the copper added to the soil sample.

Keywords: Copper sulphate; Microcalorimetry; Latosol soil; Microbe; Glucose degradation

1. Introduction

The stimulation of the microbial metabolic process of a microorganism population in soil has been shown to be very sensitive to the characteristics of the nutritional source from which only part is metabolized in a limited period of time. Disturbances promoted by the addition of any chemical to the system can be followed by calorimetric technique

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[1–3]. This procedure is favourable because all physical, chemical or biological reactions in this system are accompanied by evolution or absorption of heat [4–6].

An excessive quantity of man-made chemicals is associated, nowadays, to current agriculture practice. The use of pesticides, especially herbicides, is part of usual procedures in modern agriculture [7]. On the other hand, efforts have been undertaken to better understand the overall impact of these external chemicals added to the system as a whole or, in particular, on the living part of the soil [8–10].

The direct application of synthetic chemicals to soil can affect not only the microbial activity but also cause an overall toxic effect on the environment [8, 11]. Therefore, the residual matter becomes a pollutant and may act as a potential environmental hazard, with a great tendency to accumulate in the soil, on the ground and in the waste water [11].

The chemical-soil interaction can be followed through a series of calorimetric experiments, having the advantage that slow reactions can be followed by continuous recording of the signal for longer times, without any disturbance of the system, during the determination of a given microbial activity. For example, this activity showed a direct dependency on the amount of glucose added to red Latosol soil [5]. Therefore, direct calorimetry in a proposed system may be monitored by addition of a chemical, which is prone to give information about the thermal effects involved on the soil. Knowledge of the energy that flows in this system offers conditions to determine toxic influences on the medium. These data provide important reasons to understand ecological calorimetry [4,12,13].

In the present investigation, glucose degradation in soil was calorimetrically followed by a typical power-time curve by using a selected chemical. Copper sulphate was chosen because this compound is largely used, not only to protect trees but also fruits, normally, such as figs and grapes, in Brasilian agriculture. The disturbance and the thermal effects provoked by the increment of this chemical in the presence of nutrients is now reported.

2. Experimental

Reagents

Ammonium sulphate (Baker), glucose (Hoescht), copper sulphate pentahydrate (Carlo Erba) and other chemicals used were reagent grade. The copper sulphate pentahydrate was pulverized and dried in oven at 330 K before being added to the soil, already containing the nutrients.

Soil sample

The classified rich red soil, called red Latosol, was collected directly from the campus of the University. After removing the top surface layer, the samples were collected to a depth of 5 to 10 cm. The soil was air dried for one week and passed through a 0.59 mm sieve to separate root fragments and large particles. This soil was stored in polyethylene bags at 298 $\pm$ 5 K for at least three months before any calorimetric measurements. To characterize this soil the percentages of water and organic matter, pH, total acidity and

total cation-exchange capacity were determined as before [5]. Soil and all chemicals samples were weighted by means of an analytical balance with a precision of the order of $\pm 1.0 \times 10^{-4}$ g. The peak area values were obtained by using a manual integrator with a maximum limit error of 2%. Each measurement presented is the mean of five individual determinations, giving an uncertainty of less than $\pm 1\%$.

Equipments

The LKB2277 heat-flow microcalorimeter was used for all measurements at 298.15 ± 0.02 K. In this four-channel system the sample and reference were simultaneously introduced in a thermostated cylinder. Some calorimetric performance specifications, details of glass ampoules, their preparation to measure the thermal effect and the experimental procedures have been previously described [5,14]. For all determinations the thermal effect observed in the sample ampoule containing 1.50 g of soil, 6.0 mg of glucose, 6.0 mg of ammonium sulphate, 0.80 cm^3 of distilled water and an amount of copper sulphate varying from zero to 6.19 mg, was compared to the reference ampoule having 1.50 g of soil plus 0.80 cm^3 of water. The thermal effect associated with nutrient degradation was recorded as a function of time. This value was calculated by comparing the integrated areas of the power-time curves, which correspond to the experiment and that of the electrical calibration [5].

Calcium and magnesium in the nitric acid extractable fraction were determined with a Perkin Elmer model 5000 atomic absorption spectrometer and potassium by using a Micronal model B 262 flame photometric apparatus [5]. All thermal effects values quoted are the mean value followed by twice the uncertainty of three individual determinations [5].

3. Results and Discussion

Samples of red Latosol soil were selected for this study because it covers near 15% of the area of the State of São Paulo, including the University and, due mainly to its richness, provides a large diversity of cultivation. This fertile agricultural soil has a pH of 5.2 measured in a strong electrolyte such as 1.0 mol dm^{-3} calcium chloride in a proportion of 1.0:2.5 for soil:solution. The amount of 3.3% of organic matter per gram of dry soil was obtained by titrating the sample soil in an acid medium through a redox reaction to determine the end point. The degree of humidity of 1.3% was found for each gram of soil by means of drying the sample to constant mass. The total acidity of 3.8 corresponded to the presence of protons plus aluminum (III). This value was obtained by percolating 5.0 g of dry fine soil in air with 0.10 dm^3 of 2.0 mol dm^{-3} calcium acetate at pH 7.0. The collected eluate solution was titrated with 5.0×10^{-2} mol dm^{-3} sodium hydroxide. The amount of extractable base of 5.4 meq per gram of soil, which corresponded to be the sum of calcium, magnesium and potassium, was determined by extrating the percolated fraction of 10.0 g of the soil with 0.10 dm^3 of 5.0×10^{-2} mol dm^{-3} nitric acid. The cation-exchange capacity of 9.2 meq per 0.10 dm^3 of solution corresponded to the extracted bases and the total acidity [5].

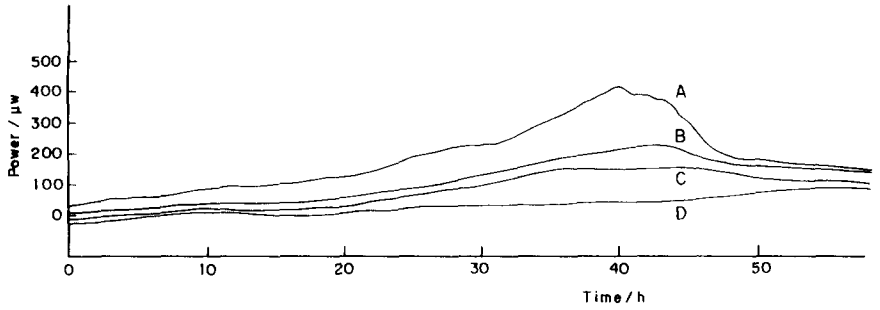

Fig. 1. Power-time curves of the microbial degradation of 6.0 mg glucose plus 6.0 mg of ammonium sulphate at 53% humidity in a sample of 1.50 g red Latosol soil, containing: (A) 0.0, (B) 1.51, (C) 3.04 and (D) 6.19 mg of copper sulphate.

The microorganism population of this red Latosol soil was shown to be very sensitive to addition of nutrients, as observed before [5]. For this kind of soil, in identical conditions, the corresponding thermal effect was always higher than for assays involving other Latosol soils [5]. The stimulus of metabolism is directly related to the amount of nutrients composed by glucose, ammonium sulphate and water. An inequivocal evidence of the microbial metabolism in degrading glucose can be visualized by comparing the power versus time plot in Fig. 1. The sample fed only with nutrients reflected in an maximum in area, which decreased to zero as copper sulphate is increased (Table 1). On the other hand, in a separate assay, a sample having only the nutrients in absence of soil also showed no thermal effect.

The thermal effect with a chosen mass of 1.50 g of soil sample was used to study the degradation of glucose, whose thermal values obtained varied with the amount of

Table 1
The influence of the mass of copper sulphate i on the maximum amplitude of the peak $t_p(i)$, the corresponding area A and $t_p(i)/t_p(o)$ ratio, for 1.50 g of red Latosol soil, fed with 6.0 mg of glucose at 6.0 mg of ammonium sulphate and 53% humidity, at 298.15 K

i/mg	$t_p(i)/h$	A/cm^2	$t_p(i)/t_p(o)$
0.0	40.0	23.9	1.00
0.77	40.9	19.0	1.03
1.51	42.6	17.9	1.07
2.01	43.0	14.8	1.08
3.04	45.0	13.9	1.13
4.25	47.2	9.6	1.19
5.28	nm	5.3	nm
6.19	nm	nm	nm

nm – not measurable.

nutrients. A standard condition of 6.0 mg of glucose, 6.0 mg of ammonium sulphate and 0.80 cm^3 of distilled water was experimentally established and gives a reasonable recorded signal. In all measurements the thermal effect produced was compared to a reference, having only the equivalent quantity of soil and 0.80 cm^3 of water. As expected, this thermal effect is very sensitive to the presence of copper sulphate, causing an inhibition of glucose degradation at 298.15 K. An illustration of this behaviour is shown in Fig. 1, where the microbial degradation of glucose is affected by the increased amount of copper sulphate. The power-time curves are always similar in shape, but shift the peak time to higher values [2,3,5]. Peak time is understood here to be the time at which the experimental curve attained its maximum amplitude. The illustration shows that the continued increase of this inhibitor causes the disappearence of the curve.

The microorganism population in the soil sample is correlated to the area of power-time curve. Thus, the increase in feeding with the proposed carbon source is followed by an enhancement in area of these curves, which represents a total metabolic activity [1,15]. The experiments reported herein demonstrate an opposite behaviour, showing that copper sulphate can cause a complete inhibition of microorganism growth. The decrease in area due to the broading of the peaks is associated with the increase in the amount of inhibitor, as shown in Table 1. These values suggest that the inhibitor takes action in the extreme situation to promote the death of microrganisms or to supress their metabolism [16].

All power-time curves, as shown in Fig. 1, change with an increase in the mass of inhibitor, resulting in a shift to longer times with a reduction of the height, becoming very broad. This same behaviour was observed before, when some pollutant substances, such as ethylmercuric phosphate, cadmium sulphate, selenic acid and iodoacetic acid were considered [11]. For this system a quantitative analysis on kinetic decomposition was fitted to a mathematical model. As the features presented by the peaks of microbial glucose degradation are similar to that found with those chemicals, the same model is applied to the participation of copper sulphate. Then, it is assumed that the change in peak time $t_p(i)$ expresses the effectof one pollutant on microbiological activity in soil and is also proportional to the change in nth power of the pollutant mass i, to give the partial derivative:

$$di^n/dt_p(i) = C$$

where C is a constant. The solution of this equation is:

$$t_p(i) = (1/C)i^n + t_p(O)$$

This model was conveniently adjusted to this inhibitor, where: i is the mass of copper sulphate (mg), $t_p(i)$ is the peak time (h), $t_p(O)$ is the peak time without copper sulphate, C ($mg^n\ h^{-1}$) and n are constants and were determined by applying a least-square treatment to the equation.

The results on microbial degradation of glucose in the presence of this chemical are summarized in Table 1. It is worth mentioning that in the absence of glucose, the sample did not cause any thermal effect. The increment in the mass of inhibitor is followed by a decrease in the area of the peak and an increase in the time for maximum intensity of the peak.

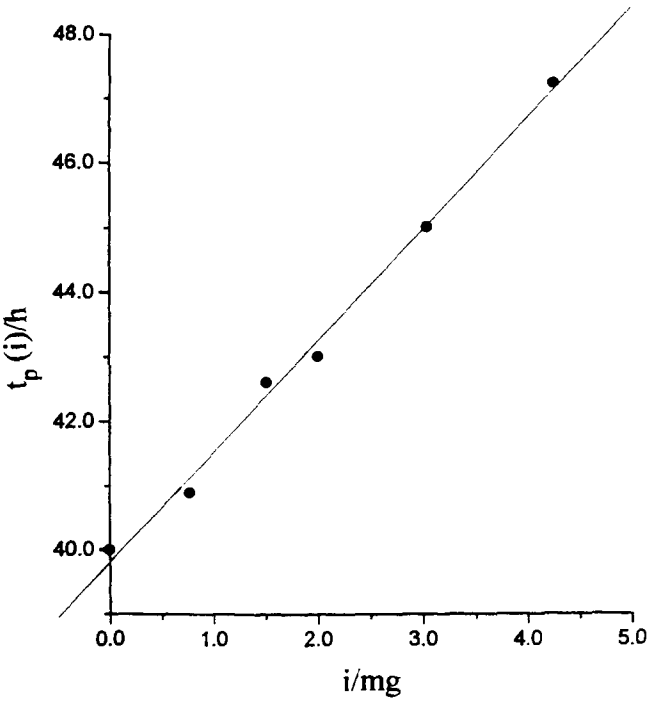

Fig. 2. Peak time $t_p(i)$ as function of mass i of copper sulphate for calorimetric curves of microbial degradation of glucose. To each 1.50 g sample of red Latosol soil 6.0 mg of glucose plus 6.0 mg of ammonium sulphate and 53% humidity were added at 298.15 K. The mass i of copper sulphate varied from zero to 6.19 mg.

From the inhibitor mass i_d, leading a peak time $t_p(i_d)$ double of that $t_p(O)$, it follows:

$$t_p(i) = t_p(i_d) = 2t_p(O)$$

$$i_d = (Ct_p(O))^{1/n}$$

and for a mass i_1 leading to a peak time differing from 1 h.

$$t_p(i) - t_p(O) = t_p(i_1) = 1\,h$$

$$i_1 = C^{1/n}$$

The mass i_d and i_1 reflect the change in the exponential curves with inhibitor in soil and the values may be regarded as the inhibitory effect of this chemical in the studied soil.

A straight line was obtained from the experimental set of values of $t_p(i)$ versus mass plot, as is illustrated in Fig. 2. This linearization showed a best fit for the data to the straight line when the n value was assumed as unity. From a least square method $t_p(O)$ and C values were calculated as 39.8 h and 0.58 mg h^{-1}, respectively. The former value showed a smooth increase with the corresponding mass values, as listed in Table 1. By applying the above equation i_d and i_1 values were calculated as 23.2 mg and 0.58 mg,

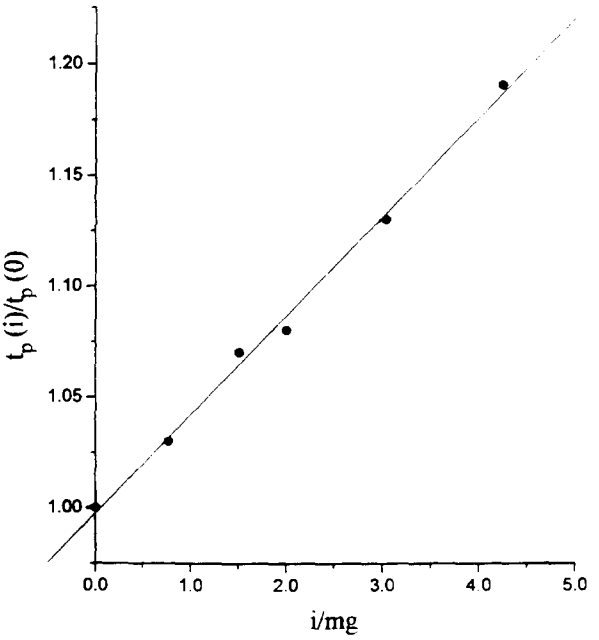

Fig. 3. The dependence of the results obtained from the variation in the peak time normalized as $t_p(i)/t_p(o)$ plotted against i. The mass of copper sulphate varied from zero to 6.19 mg.

respectively. Moreover, the relative inhibitor effect represented by the peak time normalized as $t_p(i)/t_p(O)$ and plotted as a function of mass used can also be visualized in Fig. 3.

Carbon degradation by microorganisms depends on the nature of the source and the experimental temperature [5,17], and in the present case, the results are favourable to compare because glucose was the common source chosen to be degraded at 298.15 K. However, in comparing these results with those of other chemical inhibitors [11], a different set of values is observed. These differences could be due to the experimental calorimetric conditions. For example, different soils were used, which were previously treated with the pollutant before adding water and nutrients. The procedure used before probably caused a priori some death of the microrganisms in the soil, and the humidity varied from 20 to 40%, which contrasts with the 53% used herein. This component drastically affects the shape of the peaks and consequently, the metbolic pathway [18]. Although the same nutrients were used by us and other authors [11], different amounts of the carbon source were used to feed the microorganisms, with a nutrient(mg)/soil(g) ratio of 1.0 [11], differed for our value of 4.0 and also range of inhibitor concentration. From the obtained values the n value of the pollutant concentration was calculated and varies abruptly for the series of inhibitors. In considering similar cations, n values are 0.77 and 3.60 for cadmium and mercury [11], respectively, which diverged from the unitary value for copper.

Table 2
The influence of the mass of copper sulphate ion the degradation of a sample of 1.50 g of red Latosol soil containing 6.0 mg of glucose, 6.0 mg of ammonium sulphate at 53% humidity; the respective thermal effect Q_{obs} and the calculated mass consumed m_{cons}

i/mg	$-Q_{obs}$/J	m_{cons}/mg
0.0	7.17±0.03	0.467±0.002
0.77	5.70±0.02	0.372±0.001
1.51	5.37±0.02	0.350±0.001
2.01	4.44±0.02	0.290±0.001
3.04	4.17±0.02	0.272±0.001
4.25	2.88±0.01	0.188±0.001
5.28	1.59±0.01	0.104±0.001
6.19	nm	nm

nm – not measurable

The consumption of nutrients is related to the area of the peak time, which is integrated and compared to that of the electrical calibration [5]. By using this procedure the Q_{obs} for each experiment were calculated and listed in Table 2. As expected, these values decrease with the increase of the mass of copper used, due to the inhibitory effect caused on microbial growth and metabolism in the soil.

The glucose is degraded under experimental conditions inside ampoules, which have a small hole in the top and covered with polyethylene before sealing. This arrangement permits the exchange of the internal gas formed with the external environment. In this circumstances, for the expected total degradation the final products are water and carbon dioxide [5]. By considering the enthalpy of this reaction in the condensed phase [19] and from the auxiliary data of the standard enthalpies of solution of oxygen [20], carbon dioxide [20], and glucose [21] in water, Hess' law was applied to calculate the enthalpy of the reaction in the experimental conditions as $\Delta_r H = -2762\ \text{kJ mol}^{-1}$ [5].

The amount of glucose consumed (m_{cons}) can be estimated by comparing the thermal effect Q_{obs}, caused by the fraction of glucose degraded in each experimental assayed during the microbial growth, with the proposed total degradation of one molar mass of glucose MM (180.16 g mol^{-1}), which corresponds to the thermal effect $\Delta_r H$ [5]. The thermal effect Q_{obs} is limited by the action of the pollutant over the microorganisms. These calculated values are listed in Table 2 for each experiment, by employing the equation: $m_{cons} = Q_{obs}(MM)/\Delta_r H$

The values listed in Table 2 show the exothermicity during the consumption of the glucose, which decreases with the increase of copper added in the medium. The assayed experiment without copper gave a consumption of 0.467 mg, which is nearly 10% of the original 6.0 mg, this value is in agreement with the preceding publication [5]. The progressive addition of copper caused a decrease of the glucose consumed, which is completely obstructed by 6.19 mg of copper. By considering the thermal effect Q_{obs} for each addition the respective mass consumed in each experiment was calculated and the values are listed in Table 2. The maximum amplitude of the experimental curve without inhibitor (Table 2), corresponded to the peak time of 40.0 h (Table 1) and the mass consumed was 0.467 mg of glucose (Table 2). The increase of mass of pollutant caused

a decrease in thermal effect and a correspondent shift of the time of peak maximum. For example, in comparing with the preceding value, for 3.04 mg of copper gave a peak time of 47.2 h (Table 1) and the mass consumed of glucose was calculated as 0.272 mg (Table 2). In both considered cases the consumption of glucose were 7.8 and 4.5% respectively. The addition of 6.19 mg of copper to the soil caused a total inhibition (Table 2).

The lack of quantitative data involving the influence of man-made compounds in the soil makes it difficult to compare the results cited here. However, beyond the thermal effect, it seems that i_1 and i_d could be other important parameters to consider, because they reflect the change in the power-time curve and consequently, give evidence for the inhibitory effect for a given substance. On the other hand, C varies also for each inhibitor, as observed before for other pollutants [11]. Taking into account these parameters, the purpose is to establish which one is more appropriate for predicting a better understanding of the biological interpretation of the microbial metabolism. However, other chemicals must be assayed in identical experimental conditions to give an effective comparation. For this purpose, a study of other substances is in progress.

4. Conclusion

This investigation permitted the acquisition of some values related to the microbial degradation of glucose in presence of an inorganic salt (copper sulphate). This substance causes an inhibitory effect on the degradation due to its behaviour as a pollutant agent in soil, obstructing a normal microbiological growth. The collected data from calorimetry and the consequent fit to a kinetic mechanism seems to be relevant to establish a good methodology to determine quantitatively the participation of a given pollutant in a soil microbial system. Thus, this quantitative information on soils can give considerable contributions to understanding important features related to environmental sciences.

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