ELSEVIER

Thermochimica Acta 356 (2000) 1–7

thermochimica acta

www.elsevier.com/locate/tca

Application of the metabolic enthalpy change in studies of soil microbial activity

N. Barros[a,*], S. Feijóo[a], S. Fernández[a], J.A. Simoni[b], C. Airoldi[b]

[a]*Facultad de Física, Dpto. Física Aplicada, Universidad de Santiago de Compostela, 1 5706 Santiago de Compostela, Spain*

[b]*Instituto de Química, Universidade Estadual de Campinas, 13083-970, Campinas, Sao Paulo, Brazil*

Received 14 February 2000; accepted 8 March 2000

Abstract

Some soils collected in the Amazonian State of Brazil were studied in an attempt to interpret thermodynamically a growth reaction representing balanced exponential microbial growth. The aim is to calculate and to interpret the metabolic enthalpy change per mole of glucose catabolically consumed by soil microorganisms, ΔH_{met}, to explain the changes in the microbial soil activity due to the deforestation and burning suffered by the Amazonian soils.

Locations with primary forest, pastures and agricultural plantations were chosen for sampling. Power/time curves were recorded for 1 g of soil samples supplemented with 0.2 ml of a nutrient solution containing 1.5 mg of glucose and 1.5 mg of ammonium sulphate. From the areas limited by power/time curves the total heat change of the microbial growth reaction in soil, Q_T, was calculated. The metabolic enthalpy change, ΔH_{met}, was quantified by the equation: $\Delta H_{met}=Q_T/S_0$ where S_0 is the initial quantity of glucose added.

Results showed differences in the values of ΔH_{met} calculated for the different soil samples. Soils from primary forests poor in organic matter content have higher values of ΔH_{met} than soils collected in primary forests rich in organic matter. The introduction of agriculture and pasture also caused changes in values of ΔH_{met}. It seems that the above mentioned manipulations strongly modify the microbial soil activity and the microbial population in Amazonian soils. From the values of ΔH_{met} it was possible to quantify the percentage of energy invested in growing biomass. The results showed that some plantations could be threaten by immobilization of nutrients while others could accelerate the process of desertification in that soil.

Keywords: Microcalorimetry; Amazonian soils; Metabolic enthalpy change; Soil microbial activity

1. Introduction

The study of the soil microbial activity has been always limited by the available methodologies, hence, any new method is always welcome in this area. Microcalorimetry has found few applications in soil microbiology, despite the fact that the rate of heat output is a good measure of the overall soil catabolism and that it is largely independent of the type of organism and of the intermediate reactions.

Microbial activity in soil is frequently restricted by the low levels of available nutrients and usually shows

* Corresponding author.
E-mail address: fanieves@lugo.usc.es (N. Barros)

0040-6031/00/$ – see front matter
PII: S0040-6031(00)00495-0

very slow rates of metabolism contributing to the heat output [1]. A much greater proportion of the total biomass can be stimulated into activity by the addition of an available substrate such as glucose. Such amendments result in large increases in respiration [2] and heat evolution rate [3,4]. The heat evolution rate of soil samples amended with glucose can be recorded as power time curves [5]. In this sense, Sparling [6] noted that the rate of heat output from various amended soils differed, suggesting the existence of differences in microbial metabolism. This feature can provide important information in comparative studies of soil microbial activity. Therefore, it is important to apply the method in a more quantitative way.

Microcalorimetry has been widely applied to calculate the enthalpy change of numerous metabolic, organic and inorganic reactions [7–9]. The metabolic enthalpy change of several microbial growth reactions has been also calculated [10,11]. During microbial growth, the net heat change, dQ, can be measured by calorimetry and it is equal to the sum of the enthalpy change, dH, of all reactions that occur during microbial growth. Thus, even though a growth process is composed of thousands of individual metabolic reactions, the net metabolic process comprising microbial growth can be described and thermodynamically treated as a rather simple chemical reaction [12]. It is well established in the literature that the metabolic enthalpy change per mole of glucose totally consumed during microbial growth is growth yield dependent [13,14].This dependence also occurs during microbial growth in soil. This feature could provide important data in ecological studies.

In this paper, the metabolic enthalpy change of the microbial growth reactions taking place in several soils collected in the Amazon is calculated. Soil samples from sites with Amazonian tropical rain forest, pasture and different agriculture plantations, established after burning, were used.

The introduction of pasture and agriculture in the Amazon causes changes in the microbial activity of these soils. These changes have been studied by microcalorimetry in a previous study [15]. As yet, these changes have not been well understood, hence this work is an attempt to explain those changes by the study of the metabolic enthalpy change per mole of glucose degraded by soil microorganisms, ΔH_{met}, calculated by microcalorimetry.

2. Materials and methods

2.1. Soil samples

Soil samples were collected in the Amazonian State (Brazil), 200 km away from Manaus, up Negro River, in Nova Airao. Sampling was performed during December of 1997, the dry season in the Amazonian state. Two areas in Nova Airao, named by us as T and P, were chosen for sampling. The main difference between the soil samples collected in these locations was their percentage of soil organic matter, SOM. Location P has a bigger percentage of SOM than location T. Different samples corresponding to soils from primary forests, pasture and agricultural plantations were collected in both locations P and T. Characteristics of the sampling locations are described in Table 1.

Sampling was carried out at 10 randomly chosen points from each site. After the removal of the very top layer of soil, samples were collected from a depth of 5–10 cm. All the samples from one site were mixed and sieved using a mesh size of 2 mm×2 mm. Humidity, organic matter, carbon and nitrogen percentages, as well as values of pH were calculated for all the samples. After this treatment, all samples were stored in polyethylene bags at 4°C before microcalorimetric measurements.

Table 1
Description of sampling sites

Zone P: Riverside rich in organic matter			Zone T: Inland; poor organic matter content		
Samples	Location	Vegetation	Samples	Location	Vegetation
P_{pf}	Riverside	Primary forest	T_{pf}	Inland	Primary forest
P_{i}	Igarapé	Primary forest	T_{ol}	Inland	Orange–lemon grove
P_{c}	Riverside	Cassava	T_{p}	Inland	Pasture

The number of living bacteria was determined by colony forming unities (CFU). Bacteria were isolated in soil extract medium.

2.2. Microcalorimeter

The calorimetric system used was a Thermometric 2277, Thermal Activity Monitor. This instrument is a four-channel system in which the sample and reference are introduced simultaneously in a thermostated cylinder. Some performance specifications of the LKB Thermal Activity Monitor are, detection limit 0.15 mW, baseline noise <0.2 mW, detection sensivity 0.4 VW^{-1}, working temperature 293–353 K and thermal stability 2.10^{-4} K.

2.3. Microcalorimetric measurements

All the calorimetric experiments were performed in hermetically closed 5.0 cm^3 stainless steel ampoules. One gram of soil sample was equilibrated at the temperature of the microcalorimetric measurements during 24 h. Then, the soil sample was amended with 0.2 ml of a solution containing 1.5 mg of glucose and 1.5 mg of ammonium sulphate in order to stimulate soil microbial activity and to provide the nitrogen and sulfur that microorganisms need to synthesize aminoacids. These quantities of soil and nutrient solution avoid problems related to the accumulation of CO_2 inside the ampoule [3]. One gram of soil sample amended with 0.2 ml of distilled water was used as reference to avoid the influence of the fluxes of heat derived from evaporation processes inside the ampoule on power/time curves. After this treatment, sample and reference were introduced in the microcalorimeter to register the power/time curves. All calorimetric measurements were performed at 298 K.

3. Results

The power/time curves and some properties of the soil samples used in this study, such as humidity, carbon and nitrogen percentages, as well as values of pH and soil organic matter, were reported in a previous study [15]. A clear difference was observed in the percentage of SOM between soils sampled in location T and samples collected in location P.

The power time curves recorded from samples collected in location P and location T showed the typical pattern of the microbial growth. From the areas limited by the power time curves, values of the total heat evolution, Q_T, in kilojoules per gram of soil, were calculated for all the samples. Results are shown in Table 2 together with the number of living bacteria.

As the heat evolution is proportional to the quantity of nutrient added [16,17], the metabolic enthalpy change per mole of glucose degraded by soil microorganisms, ΔH_{met}, can be calculated from the equation:

$$Q_t = \alpha(S_0 - S_t) \quad (1)$$

where Q_t is the total heat evolved to time t; S_t is the quantity of nutrient at time t; S_0 is the initial quantity of nutrient and α is the average heat evolution per unit of glucose degraded [17], which can be regarded as the

Table 2
Values of Q_T and ΔH_{met} calculated from soil samples by the microcalorimetric method together with data of the number of microorganisms and percentage of soil organic matter[a,b]

Samples	Q_T/kJ g^{-1}	ΔH_{met}/kJ mol^{-1}	No. of microorganisms/10^5 g^{-1}	SOM/%
P_{pf}	$10.82\times10^{-3}\pm1.33$	-1303 ± 161	8 ± 3	6
P_i	$9.29\times10^{-3}\pm1.80$	-1119 ± 216	6 ± 5	13
P_c	$2.98\times10^{-3}\pm0.72$	-361 ± 86	3 ± 2	6
T_{pf}	$18.67\times10^{-3}\pm1.62$	-2249 ± 196	0.5 ± 0.2	1
T_p	$9.44\times10^{-3}\pm1.86$	-1137 ± 224	53 ± 13	3
T_{ol}	$23.59\times10^{-3}\pm1.59$	-2841 ± 191	42 ± 14	2

[a] Q_T/kJ g^{-1}: Total heat change in kilojoules per gram of soil sample. ΔH_{met}/kJ mol^{-1}: Metabolic enthalpy change per mole of glucose degraded by soil microorganisms in kilojoules per mole of glucose. No. of microorganisms: number of microorganisms per gram of soil sample. SOM: percentage of soil organic matter.

[b] Data±S.D., n=3.

metabolic enthalpy change per mole of glucose degraded by soil microorganisms, ΔH_{met}. Therefore, Eq. (1) can be rewritten as

$$Q_t = \Delta H_{met}(S_0 - S_t) \quad (2)$$

Since it is assumed that glucose has been totally degraded when the power time curve returns to the initial base line after microbial exponential growth [3,17,18], it is possible to quantify ΔH_{met} from the equation

$$\Delta H_{met} = \frac{Q_T}{S_0} \quad (3)$$

where Q_T is the total heat evolution calculated from the area limited by the power time curves. Values of ΔH_{met} obtained by this method are also shown in Table 2 for all the samples.

The metabolic enthalpy change, ΔH_{met}, can be divided in a catabolic (ΔH_c) and an anabolic part (ΔH_a) in conservative reactions [19,20]:

$$\Delta H_{met} = \Delta H_c + \Delta H_a \quad (4)$$

From Eq. (4) it is possible to calculate the anabolic part of ΔH_{met}, and therefore, to quantify the percentage of energy provided by the glucose catabolism that is kept in the system in the form of biomass. Results are shown in Table 3.

Values of ΔH_{met} and ΔH_a calculated for samples P_{pf} and P_i are very similar. The value of ΔH_{met} calculated for sample P_c, -361 kJ mol^{-1}, is lower than those of P_{pf} and P_i and consequently the value of ΔH_a calculated for this sample is anomalously high. Samples collected in location T also show higher values of ΔH_{met} than samples P_{pf} and P_i. The value of ΔH_{met} calculated for sample T_p, -1137 kJ mol^{-1}, is close to those obtained from samples P_{pf} and P_i, -1303 and -1119 kJ mol^{-1}, respectively. Nevertheless, while samples P_{pf} and P_i were collected in a primary forest, sample T_p corresponds to a soil collected in a pasture.

Sample T_{ol}, collected in an orange–lemon grove, shows an anomalously high value of ΔH_{met}, -2841 kJ mol^{-1}. This result was reproducible in all the experiments performed with this sample.

4. Discussion

Changes in SOM percentages of soils that were burned and deforested and later converted into pastures and agricultural plantations, may depend on the texture of the soil, quality and availability of the organic matter. Therefore, this explains that the reasons of those changes is very difficult because the study introduces many variables. Nevertheless, the study of changes in values of ΔH_{met} can give information about the efficiency of those soils to assimilate the carbon from the organic matter and from an exogenous carbon source as glucose. This feature could be helpful to start to understand changes in SOM due to the microbial activity.

The quantification of ΔH_{met} allows to calculate the enthalpy change of the anabolic reactions. It has been reported that the enthalpy change of anabolism is low or may be negligible compared to that of the catabolic process [21]. The catabolic reaction of glucose by the respiratory metabolism results in an enthalpy change of -2814 kJ mol^{-1} [12]. Nevertheless, the metabolic enthalpy change per mole of glucose totally consumed during microbial growth is growth yield dependent. That means that a growth yield of, i.e. 0.5 g g^{-1}, very common for many respiratory bacteria, would be reflected in a decrease in the ΔH_{met} value of the total growth reaction to about -1100 kJ mol^{-1} [12]. In that sense, the contribution of ΔH_a can be substantial and it allows calculate of the percentage of energy from the glucose catabolism invested in growing biomass. Therefore, ΔH_{met} can be considered as an evaluation of the efficiency of the soil microbial community for

Table 3
Values of ΔH_{met}, metabolic enthalpy change; ΔH_a, anabolic enthalpy change and E, percentage of energy invested in the anabolic reactions of soil microorganisms[a]

Data	P_{pf}	P_i	P_c	T_{pf}	T_p	T_{ol}
ΔH_{met}/kJ mol^{-1}	−1303±161	−1119±217	−361±86	−2249±196	−1137±224	−2841±191
ΔH_a/kJ mol^{-1}	1511±161	1694±217	2453±86	565±196	1676±224	0
E/%	54	60	87	20	60	0

[a] Data±S.D., n=3.

the substrate/energy utilization. The more efficiently the microorganisms function, the greater the fraction of substrate carbon that is incorporated into their biomass and the less the carbon per unit of biomass that is lost through respiration. This feature would be reflected in the values of ΔH_{met}, that is, low values of energy dissipated as heat during the consumption of glucose would indicate small losses of carbon as CO_2. In contrast, high quantities of energy dissipated as heat in conservative reactions would mean low values of energy kept in the system in form of biomass, and therefore, higher losses of CO_2 as a result of the combustion of glucose during microbial growth.

The values of ΔH_{met} and ΔH_a calculated for samples P_{pf} and P_i indicate an investment of energy in microbial growing biomass reactions of about 54–60% of the total energy provided by the respiratory degradation of the added glucose. This means that microorganisms in those samples convert about three to four carbons of glucose in microbial biomass, and lose another three to two carbons as carbon dioxide as the result of the combustion of the glucose added. Sample P_c (collected in a Cassava plantation settled in a plot of land which was burnt 1 year before the sampling) shows a value of SOM similar to that of sample P_{pf} collected in the same zone P, but has a value of ΔH_{met}, -361 kJ mol^{-1}, much lower than those of samples P_{pf} and P_i, -1303 and -1119 kJ mol^{-1}, respectively. This sample also shows a lower number of living bacteria than samples P_{pf} and P_i, as can be observed in Table 2. All these features may be a consequence of the fire suffered by this soil 1 year before the sampling. The low values of Q_T, ΔH_{met} and number of microorganisms calculated for sample P_c are in agreement with the literature that reports a reduction of the soil microbial activity in areas that had been burnt [15,22]. The effect of burning on SOM and on the number of microorganisms in soils has been studied by several authors and is well established in the literature [23–25]. In that sense, it is striking that values of SOM and carbon percentages in sample P_c are similar to those obtained from an unburnt sample as P_{pf}. The value of ΔH_{met} calculated for sample P_c indicates that a percentage of energy of about 87% is invested in increasing microbial biomass. This fact entails that a high percentage of carbon provided from the glucose added (or perhaps from the pre-existent organic matter) is kept in soil. This feature could explain the contribution of the soil microbial activity in the Cassava plantation to the percentage of SOM and carbon calculated for that sample since the external flux of organic debris provided by the Cassava plantation is lower than that provided by primary forests. When the values of ΔH_{met} were plotted against the number of microorganisms of samples collected in location P, linear fits were obtained. The dependence of ΔH_{met} with the initial population of microorganisms in soils collected in location P has been widely discussed in a previous paper [26]. In that work it was concluded that the above dependence had nothing to do with a loss of microbial activity or an incomplete degradation of glucose, but with changes in the growth yield of the micropopulation.

Sample T_{pf}, collected in a primary forest at location T, presents a very low percentage of SOM compared to soil samples collected in primary forests in zone P. This sample shows also a very high value of ΔH_{met}, -2249 kJ mol^{-1}. This value is higher than the values of ΔH_{met} obtained from the samples collected in primary forests in zone P and it indicates higher quantities of energy dissipated as heat during glucose degradation. Therefore, most of the carbon provided by the glucose added is lost as carbon dioxide and only a small percentage, 20%, remains in soil as biomass. As there were no big differences in the vegetation of primary forests in location P and T, it is possible to assume that the quality and nature of the external organic debris did not affect the organic matter content. Values of pH of samples P_{pf} and T_{pf} are also very similar. Only the percentage of humidity in sample.

T_{pf} is smaller than in sample P_{pf}, but not so small as to introduce important changes in the kinetics of the glucose degradation [27]. Results strongly suggest that the low efficiency of the microbial degradation in this sample could contribute in some extent to the low values of SOM in sample T_{pf}. The number of microorganisms is also very low in this sample, probably as a consequence of the poor percentage in organic matter [28]. Therefore, mineralization of organic matter in the primary forests of location P is more efficient than mineralization activity in location T. The same process in location T involves higher losses of carbon as carbon dioxide.

The conversion of primary forests into pasture and arable land in zone T increased drastically the number of microorganisms (see values of samples T_{ol} and T_p in

Table 2). Sample T_{ol}, collected in an orange and lemon grove, has an anomalous high value of ΔH_{met}, -2841 kJ mol^{-1}, which caused the value of ΔH_a to be practically 0. The reason could be the easier availability of the organic matter to microbial attack in this soil. The addition of glucose activates a large proportion of the active biomass which may degrade the pre-existent organic matter to avoid problems of competition for substrate. This land was enriched with fertilizers, which causes a rapid decomposition of humus [29], probably activated by the glucose added. The heat change derived from the humus decomposition could have affected the value of the metabolic enthalpy. The introduction of fertilizers rich in organic matter could contribute to the slight increase in the SOM percentage of this soil. Sample T_p collected in a pasture shows the highest value of SOM of all the samples collected in location T (3%).The value of ΔH_{met} for this sample was -1137 kJ mol^{-1}. The microbial activity of this soil invests about 60% of the energy provided from the glucose degradation into microbial biomass. That means that about four carbons of the glucose molecule are kept in soil as biomass. Since the external contribution to the soil organic matter is very low in soils supporting pasture, and since that soil was not enriched with fertilizers, the increase in the percentage of SOM for this sample could be due to the microbial activity. It is observed that the introduction of pasture alters the microbial growth yield of the pre-existent edaphic biomass in the primary forest, changing the percentage of energy invested in microbial growth from 20% in the primary forest to 60% in the pasture, probably due to a change in the microbial composition. The high number of microorganisms observed in sample T_p compared to the values calculated for the primary forest in the same area is also striking. The reason could be the increase in the soil organic matter percentage as a result of the change in the soil microbial activity mentioned above. When the percentage of soil organic matter of samples collected in location T was plotted against the number of microorganisms, a good linear correlation was obtained as it can be observed in Fig. 1. No correlations were found between the number of microorganisms and values of ΔH_{met}, as it was found in soils from location P. No correlations were found between the percentage of organic matter and values of ΔH_{met}. The last results suggest that variations of ΔH_{met} can not be explained by the changes in the number of microorganisms in these samples.

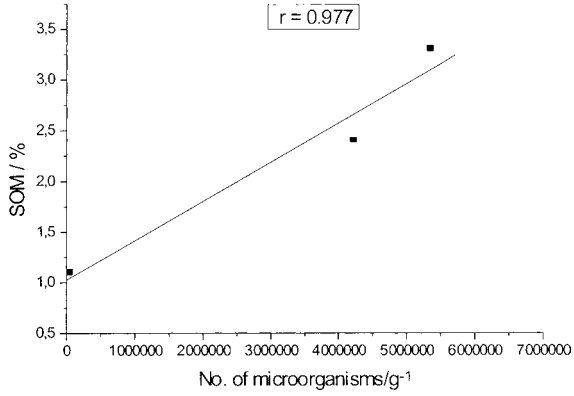

Fig. 1. Linear fit obtained plotting the number of microorganisms of soil samples collected in location T, against the soil organic matter percentage, SOM. The increased SOM percentage could be responsible for the high number of microorganisms quantified in samples T_p and T_{ol}.

Of course, we do not claim that the study of the variations of ΔH_{met} by itself may explain the variations of soil organic matter and carbon percentages in soils. But in the Amazon, those changes have not been understood yet and that feature demonstrates that the current methodologies are not enough to form a complete explanation. But we are trying to demonstrate that the use of microcalorimetry could contribute to a better understanding of those processes because it provides important information that can not be obtained by other methodologies. Results presented in this paper allow to predict the future of the agriculture plantations. The high percentage of energy kept in soil in the form of biomass in sample P_c strongly suggest a high percentage of carbon assimilation in that sample. Carbon assimilation is concomitant to assimilation of nitrogen and phosphorus, and this feature may cause problems of immobilization of nutrients that can threat the productivity of the Cassava plantation. The introduction of lemon and orange plantations in location T increases the quantity of energy dissipated as heat. This fact strongly suggests that the organic matter in that plantation is degraded at a higher rate and in a less efficient way than in primary forests. As the percentage of SOM in that location is very low, the introduction of that kind of plantations could accelerate the deserti-

fication of that soil due to big and rapid losses of organic matter as CO_2.

Acknowledgements

This work was done while N.B. and S.F. were at the University of Campinas under a grant of the Vicerrectorado de Investigación of the University of Santiago de Compostela. The authors are also indebted to the FAPESP and CNPq for financial support.

References

[1] T.R.G. Gray, Symp. Soc. Gen. Microbiol. 26 (1976) 327.
[2] J.P.E. Anderson, K.H. Domsh, Soil. Biol. Biochem. 10 (1978) 215.
[3] L. Nuñez, N. Barros, I. Barja, Thermochim. Acta 237 (1994) 73.
[4] S.A.M. Critter, J.A. Simoni, C. Airoldi, Thermochim. Acta 232 (1994) 145.
[5] K. Ljungholm, B. Norén, I. Wadsö, OIKOS 33 (1979) 24.
[6] G.P. Sparling, Soil. Biol. Biochem. 13 (1981) 93.
[7] I. Prigogine, Introduction to Thermodynamics of Irreversible Processes, Wiley Interscience, New York, 1967.
[8] H. Battley, Energetics of Microbial Growth, Wiley, New York, 1987.
[9] U. Von Stockar, I. Marison, Adv. Biochem. Eng. Biotechnol. 40 (1989) 93.
[10] Y. Ishikawa, Y. Nonoyama, M. Shoda, Biotechnol. Bioeng. 23 (1981) 2825.
[11] C. Larsson, A. Blomberg, L. Gustafsson, Biotechnol. Bioeng. 38 (1991) 447.
[12] L. Gustafsson, Thermochim. Acta 193 (1991) 145.
[13] L. Yerushalmi, B. Volesky, Biotechnol. Bioeng. 23 (1981) 2373.
[14] E. Gnaiger, R.B. Kemp, Biochem. Biophys. Acta 1016 (1990) 328.
[15] N. Barros, S. Feijóo, J.A. Simoni, A.G.S. Prado, F.D. Barboza, C. Airoldi, Thermochim. Acta 328 (1999) 99.
[16] M. Murgier, J.P. Belaich, J. Bacteriol. 105 (1971) 573.
[17] T. Kimura, K. Takahashi, J. Gen. Microbiol. 131 (1985) 3083.
[18] I. Barja, L. Nuñez, Soil Biol. Biochem. 31 (1999) 44.
[19] J.P. Belaich, in: A.E. Beezer (Ed.), Biological Calorimetry, Academic Press, London, 1980, Chapter 1.
[20] B. Birou, I. Marison, U. Von Stockar, Biotechnol. Bioeng. 30 (1987) 650.
[21] W.W. Forrest, Nature 225 (1970) 1165.
[22] R.C.C. Luizao, T.A. Bonde, T. Rosswall, Soil Biol. Biochem. 24 (1992) 805.
[23] M.A. Acea, T. Carballas, Microbiol. Ecol. 20 (1996) 33.
[24] A. Saá, M.C. Trassar-Cepeda, T. Carballas, Soil Biol. Biochem. 30 (1998) 419.
[25] M.V. Villar, S.J. González-Prieto, T. Carballas, Biol. Fertil. Soils 26 (1998) 122.
[26] N. Barros, S. Feijóo, S. Fernández, J. A. Simoni and C. Airoldi, ENTROPIE, in press.
[27] N. Barros, I. Gómez-Orellana, S. Feijóo, R. Balsa, Thermochim. Acta 249 (1995) 161.
[28] P. Prassad, S. Basu, N. Behera, Plant Soil 175 (1994) 85.
[29] M.M. Kononova, V.V. Dokuchayev, Pochovovedeniye 4 (1984) 6.