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CHARACTERIZATION OF MICROBIAL ACTIVITY IN SOIL BY USE OF ISOTHERMAL MICROCALORIMETRY

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Isothermal microcalorimetry is now established as a useful technique for the characterization of the microbial activity in soil. A brief summary of publications from this field and of instruments used in such work is presented. Several experimental parameters that can form important sources for systematic errors are discussed and it is suggested that further method work is made in this area. In most isothermal microcalorimetric investigations on the microbial activity in soil, the samples are amended with glucose. It is proposed that cellulose also will be used.

Keywords: *isothermal microcalorimetry, microbial activity, soil, systematic errors*

Introduction

Soil microorganisms are essential for the decomposition of organic matter, the cycling of nutrients and for the bio-remediation of polluted soil. They are thus of critical importance for soil fertility in agriculture and forestry and in general for our environment. For example, the microbial respiration in soil accounts for a significant fraction of the carbon dioxide released to the atmosphere and has a central role in discussions relating to the global warming [1]. Clearly, it is essential to have available a range of experimental methods by which the properties of soil, including its microbial activity, can be characterized.

This paper will focus on experimental procedures for the characterization of microbial activity in soil by use of isothermal microcalorimetry. The term 'isothermal microcalorimeter' is commonly used for calorimeters designed for measurements in the microwatt range, under essentially isothermal conditions [2]. Expressions like 'biological activity' and 'microbial activity' usually indicates some intensive property, or combination of properties, which are representative for a system of living matters. These terms are convenient to use, but are often poorly defined and it may not be possible to express observed properties in recognized units. In an isothermal calorimetric experiment it is the thermal power (heat rate, heat production rate) which is released in the reaction vessel that is measured. If the thermal power, P (sometimes the symbol ϕ is used), for a microbial system is monitored, we may therefore identify P as the 'microbial activity' of the system. For a soil sample, freed from living materials except for microorganisms, the thermal power or the microbial activity essentially originates from bacteria and fungi, of which bacteria accounts for the major part [3].

P is a strictly defined property, unit watt (W), and can be analysed in terms of well-defined thermodynamic and kinetic properties. However, soil is a very complex reaction system and it may not be possible to interpret determined P-values in much detail, but from a practical point of view P can often be used as a characteristic value for status and for changes of the investigated system.

Currently, isothermal calorimetric measurements of the microbial activity in soil are always made by use of heat conduction calorimeters, where thermopiles serve as sensors for the heat flow between the reaction vessel and a surrounding heat sink [4, 5]. The heat sink is kept at essentially constant temperature. Isothermal microcalorimeters are usually designed as twin instruments. The reaction vessel is charged with the sample and the reference vessel contains some inert material. In a twin heat conduction calorimeter it is thus the differential potential between the thermopiles that is recorded.

It has been shown that isothermal microcalorimeters are suitable for work in many areas of cell biology and a significant amount of work has been conducted on the microbial activity in different soils [1]. The technique has important advantages over other methods used in that field, but it has also some limitations. These properties are discussed in the following paragraph.

Advantages and limitations

All living systems produce heat and can therefore, in principle, be measured by some type of isothermal calorimeter, provided its detection limit is adequate. In contrast to respiration techniques, calorimetry can be used

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both for oxic and anoxic systems. For modern instruments the thermal power detection limit, reproducibility and long term baseline stability are often, but not always, adequate for measurements of the microbial activity in soil and the experimental technique is simple.

Measurements can often be made without any interference with the investigated process and the technique allows more reproducible and more accurate results than most other methods. Results can be expressed in terms of thermodynamic or kinetic properties or the instruments may be used as a general monitor for the microbial activity.

Heat measurements are non-specific, which often is an advantage when complex processes are investigated. If a specific analytical method is used to monitor a biological activity it is more likely that unknown or unexpected events will not be detected. Another advantage of isothermal microcalorimetry is that P can be recorded continuously and over long periods of time (weeks, months). However, as a consequence of the non-specific nature of calorimetric methods, it is often difficult to interpret the results in desirable detail, in particular for complex reaction systems. A related problem is the fact that results of calorimetric measurements easily are impaired by systematic errors [2, 6], as practically all physical, chemical and biological processes are accompanied by heat effects. In a later section of this paper some potential sources of systematic errors in isothermal microcalorimetric work on soil will be discussed in some detail.

In order to interpret calorimetric results for complex reaction systems, it is often necessary to obtain support by specific analytical techniques. For cellular systems in the form of aqueous suspensions, chemical analyses can often be performed simultaneously with the calorimetric measurement, for example by use of electrodes or other sensors positioned in the calorimetric vessel, by use of flow methods or by discontinuous extraction of samples, e.g. [7]. In experiments with soil such analytical techniques can hardly be used, except for the gas phase above the sample in the vessel, e.g. [8]. For the soil it is thus necessary to analyse external samples, preferably in parallel with the calorimetric experiment. It is then important that these samples are stored and handled under conditions that are closely similar to those in the calorimetric vessel, for example regarding water content and gas phase composition (CO_2, O_2).

The low sample throughput for most isothermal microcalorimeters in current use is another factor that may limit their use in characterization of microbial activity. A low sample throughput is especially disturbing in screening experiments where the effects of several substances, in different concentrations, are tested.

Typically, the equilibration time needed for a small sample (ca. 5 g or less) is in the order of 1–2 h. For a larger sample the equilibration period will be longer, as the time constant for the instrument is proportional to the sum of the heat capacities for the sample and the vessel. If very small samples could be used, as in 'chip calorimeters' [9], the sample throughput would be much higher. However, such vessels are judged to be too small for measurements of non-homogeneous materials.

The most realistic approach to overcome, or at least to reduce, the problem with a low sample throughput is to use 'multi-channel' instruments, by which several samples can be measured simultaneously. In multi-channel instruments of the heat conduction type, the different calorimetric units share the same heat sink. In some instruments all reaction vessels (channels) share one reference unit, but in other cases all channels are twin units. Some multi-channel instruments that are suitable for work with soil are commercially available.

Some isothermal microcalorimeters used in work on soil

The well-known Calvet type microcalorimeters, produced commercially by Setaram, France, have been used in several important studies of microbial activity in soil. Microcalorimeters from Thermometric, Sweden, have been used in many investigations, mainly the model called thermal activity monitor (TAM). The predecessor of TAM, a very similar instrument often called bio activity monitor (BAM), has also been used in many studies on soil and is still in use. It was based on the design described in [10] and was produced by LKB, Sweden. Another Thermometric instrument, 'TAM III', which can use up to 48 twin channels, has recently been used in soil measurements. A simpler and less sensitive multi-channel instrument, 'TAM Air', employs 8 twin channels and uses 20 mL disposable glass vials as reaction vessels. All Thermometric calorimeters are now produced and marketed by Thermal Analysis Instruments (TA), USA.

Calvet instruments used in soil research and the Thermometric instruments BAM and TAM use up to four twin channels but are normally not called multi-channel instruments. Volumes of the reaction vessels are typically in the order of 5–25 mL. Calvet instruments are in some cases equipped with 100 mL vessels, which may be adapted for exchange of the gas phase above the soil sample, e.g. Sparling [8].

Takahashi's 'multiplex' calorimeters were the first multi-channel calorimeters that were successfully used in measurements of cellular systems, including some studies of soil, cf. the brief review below. Two similar instruments have been reported: a first version using 7 channels with 30 mL disposable glass reaction

vessels [11] and a later version with 20 channels and 50 mL vessels [12, 13]. For both instruments one of the vessels is used as a reference vessel. A late version of the 20-channel instrument has been made commercially available through Laboratory of Biophysical Chemistry, Instrument Division, Keihanna, Interaction Plaza, Seikacho, Kyoto, Japan.

A mini-review

Two reviews covering investigations of microbial activity in soil by use of isothermal microcalorimetry have been reported [1, 14]. The comprehensive review by Barros *et al*. [1] include also investigations conducted by combustion calorimetry and DSC. In the review by Rong *et al*. [14] the treatment is focusing on investigations of pollutants in soil.

The following very brief summary of experimental studies will not cover all investigations that have been reported. It is rather intended to convey an impression of the different directions of isothermal microcalorimetric work on soil and of the rather few laboratories that have been involved. A special attention will be given to method developments and to work indicating possible future applications of calorimetry as analytical tools for assessment and control (early warning) of soil quality. In a few cases in the following report the calorimeters used are less sensitive than typical microcalorimeters.

Already in the late 1920s Hesselink van Suchtelen conducted a series of calorimetric investigations on microbial activity in soil; the work is summarized in [15]. Experiments were performed by use of a Dewar vessel calorimeter and results were discussed in thermochemical terms. Following that pioneering work no direct calorimetric measurements on the microbial activity in soil was reported for a long period.

In the 1970s my group conducted a series of exploratory method studies [16–18] using predecessors to the LKB-Thermometric instruments. Investigations included work on sterilization procedures, effects of long term storage at different temperatures, changes in the water content, addition of glucose [16, 17] and cellulose [16, 18]. Effects of variations of gas phase composition in the reaction vessels were also investigated [17].

In the early 1980s Sparling published results from a series of calorimetric studies on many types of well characterized soils [8, 19–21]. A Calvet microcalorimeter equipped with a large volume (100 mL) flow vessel was used. The vessel, which was charged with 10 g of soil, allowed gas exchange with the surroundings and the concentration of carbon dioxide in the gas phase above the soil could therefore be kept at a low level. Results of calorimetric measurements were compared with values determined for rates of respiration, biomass and other analytical determinations. Sparling's investigations form a landmark among the early calorimetric investigations of microbial activity in soil.

Takahashi's group has reported several significant papers on isothermal calorimetric measurements on soil [11–13, 22, 23] and of other microbial systems. From a methodological point of view their development and use of multi-channel calorimeters and their analyses of results have been particularly important. Some of their investigations point in the direction of a possible practical use of calorimetric techniques, for example in assessment of effects of fertilizers on soil [13].

Konno *et al*. have made several calorimetric studies on microbial activities in soil, one of them as early as 1976 [24]. Unfortunately, his papers are published only in Japanese. Akihiro *et al*. [25] has reported on the influence on the microbial activity from differences in soil water content and different preincubation periods. In another investigation Akihiro *et al*. [26] used a multi-channel instrument to investigate the effect of straw compost on soil.

Beese *et al*. have reported from a series of significant investigations on experimental procedures and microbial activity, where isothermal microcalorimetry, respiration measurements and other techniques were used [27–30]. The effect of a fungicide on soil was investigated [27]. Microbial properties were measured calorimetrically and by O_2 consumption [28] and the microbial activities during oxic and anoxic conditions were compared [29, 30]; in [30] the soil samples were taken from different depths and were separated into different aggregate sizes.

Results from several solitary investigations conducted by different groups, have been reported since the late 1980s. Zelles *et al*. studied a weakly acidic forest soil to which lime had been supplied [31]. A significant stimulation of the microbial activity was demonstrated by microcalorimetry, respirometry and ATP determination. Drong *et al*. [32] found that low concentrations of pentachlorophenol caused a large increase in the *P*-values, due to an expected uncoupling effect. Fradette *et al*. [33] studied the biodegradation of the herbicide 2,4-D. Soil samples, sterilized by γ-radiation, were inoculated by the bacterium *P. cepacia* that is known to degrade the herbicide to carbon dioxide and chlorine. Tancho *et al*. [34] studied the effects of pentachlorophenol and mercury chloride during a period of 28 days. Carbon dioxide formed was collected in a container connected to the calorimetric vessel and was subsequently determined by gas chromatography. Teeling and Cypionka [35] found that addition of tetraethyl lead to soil was accompanied by an increase in the thermal power, provided that oxygen was present. External analyses and calorimetric experiments with autoclaved soil showed that the decomposition pro-

cesses largely was caused by biodegradation. Tissot *et al*. [36] conducted model bio-remediation experiments with soil polluted with heavy metals and hydrocarbons. A Setaram calorimeter fitted with vessels that allowed continuous perfusion of oxygen and nutrient solutions through the soil was used.

Investigations related to the nutritional status of a soil are judged to become an area where microcalorimetric methods might develop to tools of practical importance. Several studies with this direction have been reported; e.g. [13, 14, 18, 37–40]. Dziejowski [37] used a heat conduction calorimeter equipped with a 500 mL reaction vessel charged with 25 g of soil in his investigations of decomposition of animal wastewaters in soil.

Sigstad *et al*. [38] studied three agricultural soils chosen according to their age of deforestation (3, 6 and 15 years). Correlations were made between microbial activity, organic matter and colony formation units. The effect of a worm-composite, which is used in practice as a fertiliser, was also investigated.

Very recently three experimental studies have been reported from laboratories in Wuhan, China, where a multi-channel instrument (Thermometric's TAM III) was used. Zengh *et al*. [39] investigated effects of agricultural practices on the microbial activity in different soils, whereas Yao *et al*. studied the toxic effects of cadmium [41] and hexavalent chromium [42].

The groups of Nunez and Barros, both at University of Santiago de Compostella, Spain, and Airoldi, at University of Campinas, Brazil, account for about half of all isothermal microcalorimetric investigations conducted on soil. No attempts will be made here to review all their contributions; they are well covered in the review by Barros *et al*. [1]. Many of their investigations focus on thermochemical and kinetic analyses of microbial growth curves and several of them are of significant interest from the point of view of experimental procedures. Part of the work in Nunez's group focused on connections between microbial activities and the health state of soil; for a summary of conclusions reached in those studies [43]. The effect of storage at 4°C [44] and the seasonal variation of the microbial activity were also studied by Nunez *et al*. [45]. The influence on the microbial activity in soil by the water content was investigated by Barros *et al*. [46] and by Airoldi and Prado [47]. Airoldi and Prado made also a series of investigations on the effect of herbicides on the microbial activity in soil. It was found that 2,4-D was used as a nutrient by the microorganisms [48]. When it was immobilized on silica gel the degradation was lower than for the free form, suggesting that immobilization can be a useful approach to obtain a controlled release of the herbicide [40].

Experimental and systematic errors

The thermal power for a soil sample varies with the type of soil, the site and the depth for the sampling and with the season. Further, pH, content of water, nutrients, pollutes and various organic and inorganic materials will influence the microbial activity. The measurement temperature will certainly affect the thermal power values, but no investigations conducted at different temperatures seem to be reported. Normally, the soil is homogenized and stored for some time before the calorimetric experiments, which may influence its properties. In most investigations the soil samples are amended by, for example, nutrients, pollutants or pesticides; usually in the form of aqueous solutions. In some cases soil samples are sterilized, which may initiate various non-biological processes. During the calorimetric experiments different changes of the soil material will take place, for example the concentrations of O_2, CO_2, nutrients, metabolites, water and the amount of biomass, which all may significantly affect the thermal power released in the vessel.

It was pointed out earlier that calorimetric measurements are easily impaired by systematic errors. In order to minimize such errors experimental procedures should be examined in more detail than is customary with most other experimental techniques. Except for our early work in the 1970's [16–18], hardly any method studies have been reported that refer to possible systematic errors in isothermal microcalorimetric measurements on soil. The following discussion is partly based on results reported in [16, 17].

Calibrations

The calibration of microcalorimeters is not a trivial matter [2, 6]. Calibrations are normally made by the release of electrical power in a calibration heater, which is positioned in the reaction vessel or in its close proximity. The design and the position of the electrical heater are of crucial for the accuracy of the calibration, but can normally not be modified by a user.

Normally, for a heat conduction calorimeter a significant fraction of the heat flow from the electrical heater to the heat sink will not pass the thermopile and will thus not be recorded. However, that will not cause any systematic error as long as the heat flow from the calibration heater closely mimics the heat flow pattern of the investigated process. It is important that calibrations are conducted with vessels that are identical with those used in the investigations. Further, calibration vessels should be charged with an inert material that has similar properties (amount, heat conductance, heat capacity) as the soil samples. Sterilized soil or sand, with the same water content as the

soil, can be suitable as the inert material. Chemical calibration methods are sometimes preferred [2, 6], but no such method has been recommended for vessels charged with soil.

In thermochemical investigations on well-defined chemical reactions the calorimeters should be calibrated very accurately, sometimes approaching the level of 0.01%. Soil and processes in soil are poorly defined in comparison with well defined chemical reactions and it is rarely meaningful to strive for calibration values that are more accurate than one or a few %. However, if the calibration of an isothermal microcalorimeter is not conducted with care systematic errors can easily be on the level of 10% or higher.

Sieving

Relatively small samples are used in the calorimetric measurements and in order to increase their reproducibility the soil is normally homogenized by sieving, typically using mesh size 2×2 mm. Root fragments will also be removed by that process. In some short series of experiments with a compost soil non-treated and sieved samples were compared [17]. Results indicated that the sieving process made the measurements more reproducible, without causing any significant changes in the power values. The reproducibility appeared to increase slightly if the samples were further mixed in a plastic bag. It seems that techniques of homogenization processes should be further investigated for different types of soil, in particular with respect to possible changes in their thermal power values.

Storage

In most investigations soil samples are stored for some time before the measurements, typically 3–4 months at 4°C, but in some cases soil is stored at room temperature. The storage is usually motivated by an increased reproducibility for a series of measurements conducted over an extended period of time. Mortensen *et al*. [16] reported that power values for a mor soil decreased rapidly during storage for 2 months, at 25 or 4°C. Nunez *et al*. [44] investigated a forest soil stored at 4°C and found an approximately linear decrease during a 6-month period. Further systematic investigations should be made on the effects of storage under different conditions. Should samples always be stored before the measurements?

Seasonal variation

Nunez *et al*. [45] have reported results from experiments with different forest soils, which were collected during different seasons (spring, summer, autumn and winter). Samples were stored at 4°C for up to 3 months and were amended with glucose before the measurements. Large variations of the power–time curves were observed, both with respect to type of soil and by the season at which the samples were collected.

It seems important to further investigate the seasonal variation of different soils. It may be noted that storage of a soil during a few months at 4°C corresponds to 'winter conditions' in many countries. Should the effects of seasonal variations on the microbial activity be investigated on samples that have been stored for a long time after the collection?

Sterilization procedures

Several methods have been used for sterilization of soil and there are no particular problems to exterminate all living organisms. However, ongoing non-biological reactions in the soil, e.g. oxidation processes of organic material, may still produce significant thermal powers [16]. Alternatively, and probably more important, sterilization processes might induce non-biological reactions, which together with reactions in the formed biological debris, might give significant heat effects. In our early method studies we found that heat treatment and γ-radiation can cause effects of that kind [16, 17]. Some results will be summarized here.

In a series of heat sterilization experiments with compost soil (content of organic matter was 15% of dry mass) the thermal power of the control sample was 9.0±1.7 μW g^{-1} dry mass. Samples were autoclaved for 90 min at 120°C, and the treatment was repeated three times at 24 h intervals. Plate counts showed that one heat treatment reduced the number of bacteria to 0.1% of the original value, whereas the thermal power was only reduced to 6.1±0.5 μW. After the third heat treatment there were no living cells, but the thermal power was still significantly different from zero, 2.7±0.8 μW. Similar results were obtained for a forest soil (organic matter 15% of dry mass). However, results for clayish soils, which were lower in organic material, were very different; only a slight initial increase of the thermal power was observed.

Different types of soil were sterilized by γ-radiation, 100 krad and 5 Mrad doses. Typical results for a compost soil (content of organic matter was 15% of dry mass) will be summarized here. The thermal power for the non-treated soil was about 12 μW g^{-1} dry mass; this value remained constant for at least 5 days. A 5 Mrad dose caused a complete sterilization. However, soon after the radiation was completed the thermal power was well above twice the value for the con-

trol sample. One day after the treatment the value had decreased to about 23 μW. During the following days the power decreased very slowly and was about 20 μW when the experiment was interrupted 6 days after the treatment. The samples were stored at 4°C during the observation period. In other experiments where the samples were stored at 25°C, a faster decline of the thermal power was observed. In summary, radiation of soil by 5 Mrad can cause severe systematic errors in connection with calorimetric experiments.

In some cases soil has been sterilized chemically. However, it cannot be ruled out that a chemical sterilization process might initiate non-biological reactions that will be accompanied by significant heat effects.

The results discussed above were obtained on just a few soils and no firm interpretation of the effects was made. However, the effects found are too large to be ignored and further method studies in this area are needed.

Soil and water

The water content of soil samples are often adjusted by evaporation or by addition of water. In most experiments some substance(s) is added to the soil in the form of an aqueous solution, especially glucose, fertilizers, pollutants or pesticides. A change in the water content of a soil sample will result in thermal effects, both from a change in the microbial activity [17, 46, 47] and due to changes of hydration equilibria of non-biological matter in the soil [17]. In determinations of microbial activity at different water contents by Barros *et al*. [46] and by Prado and Airoldi [47], soil samples were amended with glucose. In both studies a strong influence of the water content was found. For a red latosol soil [47] the highest microbial activity was observed at a water content of about 37%.

In some exploratory work we studied the effect of addition of water to heat sterilized soils, which later were dried at 100°C [17]. In all cases the thermal powers for the dry sterile samples were close to zero, <0.4 μW g^{-1} dry mass. Sterile water was added to the soils, which then were thoroughly mixed by kneading in plastic bags. Results of some measurements are summarized in Table 1, where the values refer to the time 2 h after start of the calorimetric experiment. It is seen that the thermal powers are much higher for the humus rich forest soils than for the field soils and that the values for the forest soils are nearly proportional to their content of organic matter. Results suggest that the thermal effects largely are due to hydration of the organic materials, but it is possible that the heat treatments had affected its original properties.

Very large thermal powers, about 1 mW g^{-1} dry mass, were found for heat sterilized samples if the mixing after addition of water was not made carefully. Such effects decreased rapidly with time, but could be observed during several weeks. It is concluded that addition of water or aqueous solutions to soil may cause very significant systematic errors if mixing processes are not conducted with great care.

Table 1 Thermal powers from heat sterilized soils, after drying at 100°C, followed by addition of water to 15% of wet mass (data from [17])

Type of soil	Organic matter/%	Thermal power/μW g^{-1}
Clayish field soil	5	7
Sandy field soil	4	2
Beach and oak forest soil	14	28
Spruce forest soil	23	48

In most isothermal microcalorimetric work on soil the samples have been amended with glucose and often also with other materials. Additions are normally made by use of aqueous solutions, but glucose has also been added as a finely ground powder. Sufficient information about the employed mixing procedures is rarely found in publications. Were hydration equlibria in the soil samples established before the calorimetric experiments commenced?

Long term experiments

It is often desirable to conduct measurements over extended periods of time, weeks or months, which often is possible with respect to the baseline stability of the instruments. However, usually the calorimetric vessels are hermetically sealed and due to soil respiration concentrations of O_2 and CO_2 will then decrease and increase, respectively, in the soil and in the gas phase above the soil. Eventually this will result in O_2 deficiency and/or a harmful accumulation of CO_2 [17]. There are some experimental procedures by which the O_2/CO_2 problem can be managed. The most straightforward strategy is to work with the largest possible volume of the gas phase in the reaction vessel. In a typical case a 5 mL vessel is charged with about 1 mL of soil and is amended with glucose. Under such conditions measurements can usually continue for about 10 h before the conditions in the vessel start to deteriorate.

Sparling [7, 19, 20] used a Calvet microcalorimeter equipped with a 100 mL flow vessel, which allowed gas exchange with the atmosphere. Concentration of CO_2 did not exceed 0.08%, which was judged to be acceptable. However, no further experimental details were reported. It should be noted that thermal powers from evaporation/condensation effects can be very large, even for modest flow rates, if the water activity of the incoming gas and the soil are not closely identical [6].

If such effects are constant during the experiment they will cause a constant offset of the baseline that can be corrected for, provided that proper control experiments are made. However, it may not be possible to keep constant conditions during extended experiments, a week or more, in which case corrections can hardly be applied.

Tissot [36] perfused oxygen and nutrient solutions through a 12 mL reaction vessel, which partly was charged with soil. No details were reported on possible disturbances of the calorimetric signal caused by the gas flow.

In our early work we used a novel ampoule technique to avoid harmful O_2/CO_2 concentrations during long term experiments [17, 18]. Soil samples were enclosed in thin-walled cylindrical plastic (polyethylene) ampoules, where top and bottom consisted of discs made from 1 mm silicone rubber, which has a very high permeability for O_2 and CO_2. The plastic ampoules fitted snugly into the calorimetric steel vessel, which was hermetically sealed during measurements. During an extended experiment the plastic ampoules were kept outside the calorimeter; only during short measurement periods they were enclosed by the steel vessel. If the plastic ampoules were charged with soil amended with glucose, no change in concentrations of O_2 and CO_2 were observed after 48 h. However, when an identical soil sample was enclosed in the plastic–steel assembly, a CO_2 level inhibitory to aerobic metabolism was reached within hours. A significant decrease of the O_2 concentration was also observed. The CO_2 accumulated leaked out within 1 h, when the plastic ampoule was freely exposed to air.

However, there is a significant problem with the technique: the very high permeability for water vapour in silicone rubber. That problem was not considered in our work, but it can be handled if the plastic vessels with soil are stored in equilibrium with air, at the same water vapour pressure as for the soil, except for the short calorimetric measurement periods. Another problem will occur if the thermal power for the sample will change fast and in an irregular way during an extended experimental period. In such cases it might be difficult to construct an accurate thermal power curve from results of the intermittent measurement periods.

Further method work on the gas phase problem should be made, especially concerning gas flow techniques and gas permeability problems. It would be desirable to have available a material with high permeability for CO_2 and O_2 combined with a relatively low permeability for water vapour.

Why only glucose?

Normally there are a large number of microorganisms in a sample of natural soil. However, in general only a small fraction of them are metabolically active, due to a shortage of suitable energy substrates or mineral nutrients. If soil is amended with glucose and necessary mineral nutrients are available, there will be a fast increase of the thermal power, partly due to an activation of the initial population of microorganisms and partly due to microbial growth. Many important investigations have been made in this field and much of the thermochemistry and the kinetics of these processes have been clarified by isothermal microcalorimetric measurements, usually in combination with respirometry [1].

There can also be another and more practical reason why so many studies have been made with soil amended with glucose. As the thermal power of natural (non-amended) soil is low, typically in the range of 10 $\mu W\ g^{-1}$ dry mass, it can be difficult to characterize precisely the effects of addition of various materials, in particular if the amendments will decrease the microbial activity. Glucose will in such cases serve as an 'amplifier', increasing the difference between thermal power curves for the inhibited system and the control.

Glucose does not exist in natural soil at concentrations used in the calorimetric experiments. It therefore seems important to extend those investigations to materials that are closer to those available in nature, especially different kinds of well-defined cellulose. Except for our first exploratory method work [16] no such studies seem to have been reported. Figure 1 summarizes experiments where a mor soil is amended with a mixture of nutrient salts, cellulose powder and a mixture of the salts and cellulose. No significant difference was observed between the non-treated soil and soil amended with salts. When cellulose was added a slow increase was observed after an incubation period of about 10 days, whereas for soil amended by salts and cellulose a large increase of the thermal power curve is seen after

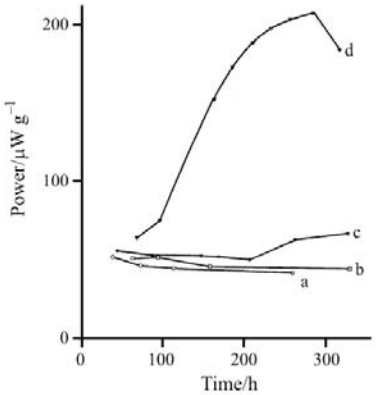

Fig. 1 Power–time curves for a mor soil amended with salts and cellulose. Salt mixture: $NaNO_3$ (0.1%), $MgSO_4 \cdot 7H_2O$ (0.1%), K_2HPO_4 (0.2%); a – no treatment; b – salt mixture added, c – cellulose powder (2%) added, d – salt mixture and cellulose powder added. The water content was the same for all samples (34%). Measurement temperature was 25°C (Fig. 1 was derived from [16])

a lag period of about 3 days. No attempts to interpret the results were made, but it seems that different types of experiments with soil amended with well-defined samples of cellulose should be explored.

Conclusions

Isothermal microcalorimetry is now established as a useful technique for the characterization of the microbial activity in soil. A significant amount of work has been reported, but presently only a few scientific groups are active in this field. It is judged that with the use of multi-channel instruments their number will soon increase and it is predicted that isothermal microcalorimetric techniques will develop to important analytical tools in applied soil microbiology.

Discussions in this paper have referred to several experimental parameters which can form important sources for artefacts and systematic errors. It is felt that further method work in this area is needed and it would be valuable if experimental procedures were reported in more detail.

In most isothermal microcalorimetric investigations on soil the samples are amended with glucose. It is proposed that experiments are extended to the use cellulose as an added energy substrate.

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