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Calorimetry in ecology

Ursula Reh
Institut für Biochemie und Molekulare Biologie, Technische Universität Berlin (Germany)

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INTRODUCTION

> "Though the organisms may claim our primary interest, when we are trying to think fundamentally we cannot separate them from their special environment, with which they form one physical system" (quoted by Chapman [1]).

In this way, Sir A. Tensley formulated the interest in the new field of "Ecosystems" in 1935.

In a thermodynamic sense, organisms or populations are open systems exchanging matter, energy and information with their environment. These activities are in all cases connected with a flow of heat, because the Second Law of Thermodynamics requires a decrease of order in the whole system, i.e. organisms plus environment. Thus, life as a metabolic process creating order must be connected with reactions imposing increasing disorder on the system.

Heat directly related to the enthalpy change (ΔH) may be measured by calorimetry. Energy bound in organic matter by metabolic processes can be determined as heat Q by combustion calorimetry and DSC. Thus, the combination of these three calorimetric methods makes it possible to calculate the total energy flow through an ecosystem.

Microcalorimetry as well as bomb calorimetry are well established methods in all branches of ecology. Ecosystems of soil or water, of plants and animals, of special faunal and floral populations, of life and particularly microbial life at different temperatures, with different nutritional and oxygen supplies or at different geographic gradients or elevations have been investigated.

The ecological aspects of the microbial production of drugs and the decomposition of xenobiotic pollutants, of waste water treatment and of conversion of renewable biomass into fuels are mentioned as examples from publications of the last ten years.

SCOPE OF CALORIMETRIC INVESTIGATIONS

In the following sections, calorimetric experiments on the various parts of an ecosystem are described, starting with soil and its different con-

stituents, and with water and sludge. They are followed by plants as an essential part of an ecosystem, by animals and by complete populations. Data concerning environmental conditions, nutrition and toxicants complete this survey.

SOIL

The objects of investigation can be natural soils or their covering litter, agricultural soils, or aquatic sediments, from nearly all geographic regions.

Dealing with these very different soil ecosystems, interest focussed on the following main aspects in calorimetry:

(i) Characterisation of organic matter and, especially, humic substances;

(ii) Characterisation of the decomposition processes of the organic matter, mainly obtained by combustion calorimetry, DSC or DTA;

(iii) Estimation of actual and/or potential activities of bacteria, fungi and microfauna or unspecified activities in normal, acidified or limed soils and, deduced from that;

(iv) Estimation of the biomass in soil by microcalorimetry;

(v) Determination of seasonal trends in metabolic activities.

Organic matter and humic substances

The characteristics of humic substances in soil (A_h horizons) were correlated with different kinds of vegetation. A higher degree of condensation and thermal stability, and a generally higher carbon content were found in soil samples under grass than in those from soils under pine, sedges and cultivation [2]. More thermostable humic acids and fulvic acids were also found in industrially and environmentally polluted soils and sediments [3].

Weddellite (calcium oxalate dihydrate) concentration in sediments rich in organic matter characterised by DTA were used to determine directly the percentage of organic matter preserved in the sediment [4]. Also, the weathering stages of soils were related to the status of natural vegetation [5].

Decomposition processes of organic matter

Thermoanalytical methods were used to determine energetic characteristics of decomposing processes in mixed forest litter [6], and in organic material derived from several plant species [7] or individual litter species, e.g. *Prunus serotina* and *Carpinus betulus* leaves, and *Pinus sylvestris* needles [8] and *Festuca arundinaceae* litter [9].

As in litter, organo-mineral complexes in soil were also characterised by two typical exothermic reactions at 300 °C, derived from plant debris and

inorganic soil material, and at 400–450 °C, from soluble humic substances plus inorganic soil material [10]. Reh et al. [8] attributed the reactions at 350 °C to the holocellulose complex and those at 400–450 °C to lignin and its products of decomposition.

Microbial activities in soil

Several authors have investigated metabolic activities in soils or aquatic sediments by microcalorimetry. A "total" activity in natural aquatic sediment samples from aerobic environments was measured by Gustafsson and Gustafsson [11]. The heat evolved by sediments decreased with depth. The total heat flux is an indication of the rate of degradation of the potential chemical energy originally fixed by photosynthesis and represents the benthic energy flow [12]. Data from sediments show variable correlations between heat production determinations and other methods describing biological activity (such as ATP concentration and the activity of the electron transport system). As expected, long-term storage of sediment resulted in a decrease in the rate of heat production, possibly due to exhaustion of food substances or of electron acceptors, or to changes in microbial composition [13].

A decrease in activity of soil profile with depth was seen with increasing anaerobic processes and depended to a large extent on the type of habitat and soil moisture. Therefore respirometric measurements were combined with direct calorimetry [14,15]. The decrease in heat production in stored soils mentioned above was also followed microcalorimetrically and respirometrically by Sparling [16]. He determined 21.1 J per cm^3 CO_2 evolved and deduced a largely aerobic metabolism for the soils investigated.

The highest activities in a spruce forest were measured by heat output, respiration and ATP concentration in the upper horizons; most values decreased gradually with depth. In addition, humidity derived either from acid precipitation or from irrigation, and liming stimulated the activity, particularly in O_{f2} horizons and, to a smaller extent, in deeper horizons. In the O_l and O_{f1} layers, acid precipitation reduced bioactivity [17]. In general, removing the water limitation and mixing the natural soil layers stimulates the activity [18].

Besides the measurement of actual activities without any additives to the soil, the potential activities of microorganisms and maximal heat production (and/or respiration) were investigated by removing the limitations on the available energy sources by addition of carbohydrates. Yamano and Takahashi [19] studied the effect of different sugars on microbial activity in soil and found the following order: D-glucose (92 kJ mol^{-1}) > sucrose > lactose > D-fructose > D-galactose > D-mannose (64 kJ mol^{-1}). Kimura and Takahashi [20] found a linear relationship both between heat evolution and the amount of glucose degraded in soil (1287 ± 52 kJ per mol glucose) and

between heat evolution and viable cell counts determined for bacterial cells (6.7 pW per cell) in pure cultures. Sparling [16] measured an actual activity of 20.4 mW per g biomass carbon for freshly collected mineral soils and a potential activity (0.5% glucose w/w added) of 188.2 mW per g biomass C.

The effect of acid precipitation on microbial activity was also investigated by Ljungholm et al. [21]. The heat production of acid-leached soils was smaller than that of water-leached control soil. An attempt to restore acidified soils first by addition of an aqueous solution of sodium hydroxide, then by supplementation of an energy source (cellulose powder) and new inoculation, resulted in an increase in microbial activity. Of course, a true "restoration" could not be attained by this procedure. In addition, the heat dissipation with different glucose concentrations, with increasing water content in soil, with depletion of O_2 or excess of CO_2 and the heat effects following sterilisation procedures, were investigated by Ljungholm et al. [22,23].

Estimation of biomass in soil

Sterilisation effects were determined by Sparling [24]. He estimated the biomass in the soil by the amount of carbon mineralised after chloroform fumigation and by the rate of respiration in substrate-amended soil, compared with untreated soils. He found a consistent relationship between the biomass and the rate of heat output from freshly collected and amended mineral and organic soils, with a linear fit using logarithmic transformed data:

$$\log(\text{biomass C}) = 0.6970 + 1.025 \log(\text{heat output})$$

where biomass C is given in g C per g ww and heat output in W per g ww at 22 °C [16].

Seasonal trends in metabolic activities

Seasonal trends in metabolic heat production and their oxidative counterparts were investigated by Tournie and Lasserre [25]. They found typically unimodal microcalorimetric curves in marine microcosms at the water–sediment interface in summer. In winter, the power–time curves are typical sigmoidal. Spring and autumn responses behaved heterogeneously, varying from a summer to a winter-type.

Soil heat flux and water distribution were analysed in an agricultural soil by Pikul et al. [26]. They measured a daily soil heat production ranging from 710 to 300 kJ m^{-2} and an additional daily heat flow of about 1000–2400 kJ m^{-2} owing to evaporation of water layers between 0.42 and 0.98 mm.

WATER AND SLUDGE

Aquatic environments are less stable in composition than soils. Seasonal circulation of water (see, also, section on soil), fluctuations in nutrition and salt content, and influxes of waste water, which induce reactions of microorganisms, have all been investigated. Aquatic sediments and water–sediment interface ecosystems are discussed under soil.

An international workshop in 1986 considered the determination of microbial activities in the carbon cycle of aquatic ecosystems [27]. In particular, the effect of high molecular weight organics on the activity of the epilithic microflora was measured calorimetrically [28].

Fiddler crabs (*Uca pugilator*) are excellent osmoregulators, adapted to salinity changes by regulation of their hemolymph concentration. Their heat production after adaptation showed a significant increase at water salinities diverging from normal [29].

The degradability of cellobiose, soluble starch, skimmed milk, carboxymethylcellulose and olive oil emulsion in primary sludge and digested sludge, taken directly and after storage, and tap water from a garbage dump, as well as the adaptation of the system to the respective substances, were investigated by Redl and Tiefenbrunner [30].

The addition of toxic compounds (cyanide, cadmium, chromium, copper and phenol) to activated sludge mixed with synthetic biodegradable effluents in batch and continuous recycling processes were studied in a flow microcalorimeter by Fortier et al. [31]. The heat flux measured was in general accord with the data obtained by respirometry.

PLANTS

As mentioned in the introduction and as a shown in the section on soil, calorimetric investigations are divided into two sections: measurements of metabolic activity by microcalorimetry and characterisation of the substrate (here, plant material) and its energy content by combustion calorimetry, DSC and/or DTA. In this context, the use of plant material for conversion into fuels has often been discussed in the literature as an alternative energy source. Unfortunately, the superseding of food production (e.g. *Sorghum*) or the irresponsible destruction of forest as an important climatic factor and ecosystem, have not been discussed by any author.

A combination of these two calorimetric approaches was attempted by Wise et al. [32] by measuring bioconversion efficiency. Aquatic plants (*Lemna* sp. and *Hydrilla verticillata*) and marine algae (*Gracilaria ceae* and *Ulva lactuca*) were anaerobically fermented to methane as part of an evaluation of these biomasses as energy resources. The conversion efficiency was based on energy output, determined as methane (unfortunately not as heat) and energy input, given as caloric values of the biomass. The

results indicated that cellulose was converted and that the residual higher energy components such as lignin were non-biodegradable under the chosen conditions.

Comparable to the latter process is ethanol production from *Sorghum* biomass by anaerobic yeast fermentation, yielding up to 3418.3 litres of ethanol per ha [33].

Other authors have also emphasised the investigation of cellulose degradation. Nakasaki et al. [34] composted sewage sludge by adding wood chips and measured the microbial decomposition of cellulose material by the concentration of water soluble sugars (calorimetric method of Dubois). The two sources of cellulose, sewage sludge and wood chips, were decomposed by thermophilic bacteria and fungi. But the exclusive assignment of sludge decomposition to bacteria and of wood decomposition to fungi in a mixture of both substrates asserted by the authors, seems questionable.

Determination of the anaerobic microbial decomposition of straw samples by microcalorimetry, gas chromatographic analysis of the metabolic products, as well as the interaction between the endogenous flora of straw and the added inoculum were discussed by Partos and Fardeau [35].

The degradability of some celluloses of varying degrees of crystallinity was investigated microcalorimetrically by Dermoun and Belaich [36]. They found that microcrystalline celluloses were not completely utilised, whereas amorphous cellulose was easily metabolised. The heat evolved by decomposition of cellulose was -5.86 kJ g^{-1}, a value similar to that obtained with glucose in culture. Parameters of thermogravimetric analysis (TGA) were positively correlated with crystallinity in pyrolysis of corn husk residues under nitrogen atmosphere. DSC and TGA profiles reflected the main constituents by exothermic effects at 238 °C (hemicellulose) and 317 °C (cellulose) [37].

Energy losses, biological efficiency, and the energy fixed in fruit bodies and in proteins of the wood-rotting fungus *Pleurotus ostreatus* were calculated by Ginterova and Lazarova [38]. An attempt to characterise sound and fungally degraded wood by DSC was made by Reh and coworkers [39–41]. Hemicellulose, cellulose and lignin components were detected as discrete exotherm peaks of combustion at defined temperatures (300, 350 and 400–520 °C) in sound wood (*Betula* sp., *Fagus sylvatica*, *Picea abies* and *Eucryphia cordifolia*). Characteristic changes were obtained from birch wood samples degraded by the basidiomycetes *Fomes fomentarius* and *Piptoporus betulinus*. From these degradations, sequences for white- and brown-rotted wood were postulated.

The ecological efficiency and the energy budget of a natural grassland were studied by estimating the caloric values of 5 components. The net energy production was 9759 kcal m^{-2} a^{-1} (40861 kJ m^{-2} a^{-1}), the total loss of energy was 3670 kcal m^{-2} a^{-1} (15366 kJ m^{-2} a^{-1}) and the ecological efficiency of the community was 1.17% [42]. The thermal energy

TABLE 1

Average energy values (kJ g^{-1})

	Wood	Bark	Leaves
Carpinus betulus L.	−19.10	−19.11	−19.77
Acer campestre L.	−18.19	−18.70	−19.64
Quercus cerris L.	−19.01	−19.56	−20.65
Quercus petrea Liebl.	−18.12	−18.25	−19.09

of certain grass species or their by-products (rice straw, Bermudagrass and sugarcane bagasse) used for rice drying were measured by Verna [43].

The energy potential of *Euphorbia esula* as a fuel crop was evaluated. The oil content of 6.8% had a calorific value of 10.0 kcal g^{-1} (42 kJ g^{-1}) and the whole plant biomass contained 4.4 kcal g^{-1} (18 kJ g^{-1}). Thus, *Euphorbia* produces 4 times more energy per year than wheat straw [44].

Based on caloric values of different tree components of a *Platanus occidentalis* plantation in the range of 4.49 kcal g^{-1} (18.8 kJ g^{-1}) for stem wood to 4.75 kcal g^{-1} (19.9 kJ g^{-1}) for stem bark, the total energy yield for a 1 ha plantation (1200 trees, 20.3 tons of above-ground biomass) was computed as 9.22×10^7 kcal. The storage efficiency was calculated to 0.55% [45]. The energy values of wood, bark, first-order branches, twigs and leaves of nine hardwood species (*Acer rubrum, A. negundo, Cercis canadensis, Fraxinus pennsylvanica, Liquidambar styraciflua, Liriodendron tulipifera, Plantanus occidentalis, Prunus serotina* and *Quercus nigra*) were determined. Intraspecies differences between tissues averaged at 4.79 kcal g^{-1} (20.1 kJ g^{-1}) and were greater than the relatively small but significant differences between species [46]. Significant to very significant differences in energy content between wood, bark and leaves of different trees were also described by Oszlany [47] (see Table 1).

The average caloric value of 40 fast-growing trees was found by Abe [48] to be 4.70 kcal g^{-1} (19.7 kJ g^{-1}). The measured elemental composition of bark was almost the same as that of wood: C, 50%; H, 6%; O, 44%. Dulong's equation for estimating the calorific value of solid fuels from their elemental composition was corrected for its application to bark and wood. The combustion characteristics of rubberwood and eighteen common Malaysian wood species were compared by Tan and Stott [49] using DTA and TGA. The energy content of *Picea abies* Karst. wood was studied by Ivask [50]. He found that the early wood layers do not differ in caloricity from the late ones, whereas the width of annual layers and their caloricity were correlated. This was also confirmed by Reh and Kraepelin [51] for wood samples of *Picea abies* with a mean of 5.5 rings per cm radius and a total heat release of 8.22 kJ per g dw, as compared with 22 rings per cm and 9.74 kJ per g dw.

Colin and Jones [52] discussed research in pollination ecology. They determined the caloric content of pollen of several different species and observed significant differences between wind-pollinated dicots versus monocots and gymnosperms. Differences were also found between two tribes of Asteraceae providing additional evidence for independent evolution of the two.

The thermodynamic parameters of air-classified starch and protein fractions of eight legume flours were investigated by Sosluski et al. [53]. The enthalpies of gelatinisation and denaturation aggregation were highly variable, not specific for species, and appeared to be primarily determined by environmental conditions during seed development.

Investigations on the comparative allocation of biomass energy and nutrients in *Verbascum thapsus* and five *Solidago* spp. were performed by Abrahamson and Caswell [54]. The strong correlation between biomass and energy allocation in *Solidago* led the authors to the conclusion that calorimetry is not necessary to determine an energy allocation pattern. Jolls [55] contradicted this opinion and pointed out that caloric values differ for phyletic and ecological categories, for plant species, tissues of the same plant, and within a tissue for different seasons, microhabitats and elevations. She explicitly warned against exclusive use of biomass when resource allocation is the basis for comparing populations of plants.

ANIMALS

Only a few examples of calorimetric and ecosystemic investigations on animals shall be mentioned here (see section on soil). A significant store of energy in the deep sea by Holothurians was described by Walker et al. [56], who measured the total body caloric biomass at depths of 1010, 2000 and 4000 m (0.49, 3.69 and 0.25 kJ m^{-2}). The total body calorific value varied from 24.26 to 25.43 kJ per g ash-free dry weight. Tissue heat content was determined from biochemical data and microbomb calorimetry.

Microbial heat production from decomposing excrements of different animals was investigated by Bolouri and Lamprecht [57]. The possible energy produced from the excrement of one cow was estimated to 225 J kg^{-1} wet weight and 11.250 MJ per day. Compared with its heat of combustion (20 kJ g^{-1}) this was only a small profit. But the authors argued for a possible further use of compost.

The anoxic heat dissipation of *Lumbriculus variegatus*, measured by direct calorimetry, is reduced by up to 85% relative to aerobic rates. The decrease of anoxic heat dissipation coincides with the disappearance of peristaltic movements and the cessation of defecation when the gut is half emptied. Anoxic catabolism of glycogen may not fully explain the directly measured rates of heat dissipation under environmental anoxia [58].

Öschger [59] calculated directly the reduced glycogen consumption of marine invertebrates under anoxia using calorimetric measurements. These measurements on bivalves showed that they reduced their metabolism by up to 40% during the first day of oxygen lack when they switched to an anaerobic metabolism. After prolonged anoxia, energy release decreased to less than 1% of aerobic rates. This behaviour may help the organisms to withstand adverse environmental conditions.

POPULATIONS

Because of instrumental conditions, microorganisms, micro-, meso- and macro-faunal groups of organisms, as well as communities of small insects are the preferred subjects of calorimetric investigations.

Successive phases of mesophilic, thermotolerant and thermophilic activities of microbial populations in pig, cow, horse- and poultry manure, household waste, spruce wood, grass and hay were measured by Bolouri et al. [60].

Attached mixed autotrophic–heterotrophic microbial communities in rivers and their energy sources were investigated by Lock and Ford [61]. The influence of added nematodes (*Diplolaimella chitwoodi*) and polychaetes (*Capitella capitata*) to microbial batch cultures was tested by Pamatmat and Findlay [62]. The nematodes prolonged the lag phase and prevented the microbial population from reaching the peak metabolic rate without nematodes. Polychaetes maintained microbial metabolism and population at a relatively steady state.

The energy flow-through and the heat balance in an ant hill (*Formica polyctena* Foerst.) was determined by Bachem et al. [63]. They found that 86% of the heat produced in the nest was contributed by microorganisms (0.45 mW per g nest material for the periphery and 0.65 mW g^{-1} for the centre). The higher microbial activity in the centre of the hill was explained by the cultivating activity of the ants, fertilisation by faeces and finer granulation. Coenen-Stass et al. [64] also considered the question of heat production of ants, pupae and nest material of an ant hill of *Formica polyctena*. Calorimetric and manometric data were used to estimate their possible contribution to the heat balance of the nest hill. The origin of the major heat production from microbial activities was confirmed.

Energetic and ecological relationships among six coexisting mayfly species (*Ephemerella* spp.) were investigated by Sweeney and Vannote [65]. A hypothesis was presented to explain the interaction of temperature, insect development processes and physiology in determining both the timing emergence and the resultant size and fecundity of individuals.

Heat production of members of different bee castes (*Apis mellifera carnica*) and thermoregulation of the whole colony during summer and winter were measured by Fahrenholz and coworkers [66–68]. Individual

worker bees show a strong positive correlation of the weight specific heat production rate with age. Increasing numbers of workers together in a group reduced the heat production drastically, so that the group of 12 bees dissipated less heat than one isolated animal. Addition of a queen or of bee brood to a group of six workers also lowered the heat output. These effects could be described as socially conditioned.

ENVIRONMENTAL CONDITIONS

Temperature

The living conditions of microorganisms, plants, animals and man at different temperatures, and their contribution to altering their own environment by heat, are discussed in the following section. The effect of cooling below room temperature to minus degrees centrigrade on animals and plants has been investigated by several authors.

Using DSC analysis, an anti-freeze protein was detected in larvae of the common mealworm (*Tenebrio molitor*) by Hansen et al. [69]. Low molecular weight cryoprotectants and an ice-nucleating agent in the plasma, controlling freezing of extracellular water, were found in the wood frog (*Rana sylvatica*). In effect, ice was restricted to extracellular spaces, undercooling minimised, cross-membrane osmotic stress during freezing gradually applied and freeze concentration of cells can be slowed and regulated [70].

The effect of environmental temperature (24, 20, 16, 12 and 10 °C) on extra thermoregulatory heat production, maintenance heat production, and protein and fat retention in breeding boars was investigated by Kemp et al. [71]. Above a critical temperature of 20 °C, protein and fat were retained; below this temperature, protein and fat gain decreased, and at 10 °C the boars lost fat.

To examine the suggestion that positive feedback plays a role in temperature regulation of the nine-banded armadillo (*Dasypus novemcinctus*), changes in heat loss (direct calorimetry) and heat production (indirect calorimetry) were measured in thermoneutral and cold environments. Mercer and Hammel [72] demonstrated that the rise in core temperature was accompanied by a positive heat storage in the body and that core thermosensitivity was similar to that in a variety of other homeothermic mammals.

As most published investigations on the cold hardiness of plants deal with freezing damages and their prevention in fruits and vegetables, belonging more to an agricultural than ecological field, only one example will be mentioned here. Thermal hysteresis, freeze–thaw reactions and cold hardiness of acclimated and non-acclimated flower buds of six azalea species (*Rhododendron* spp.) were measured by pulse nuclear magnetic resonance spectroscopy and by differential thermal analysis [73].

Thermotrophic properties of thermophilic, mesophilic and psychrophilic blue-green algae grown at high, room and low temperatures were studied by differential scanning microcalorimetry. Chang and Berns [74] were able to correlate the relative thermal stability of some algae with an isolated biliprotein, detectable in thermograms of these organisms as an endothermal denaturation peak in the range of 50–70 °C.

The thermoregulatory reactions of animals under heat stress were also investigated. Heat loss and thermoregulatory responses in rats acclimated to continuous heat exposure of 24, 29.4 and 32.8 °C were measured by Shido and Nagasaka [75,76]. They found a suppression of metabolic heat production during intraperitoneal heating, especially at 32.8 °C, and that the core temperature level was lowered as the acclimation temperature increased.

The energy metabolism of lactating cows at 15 and 30 °C was investigated by Nauheimer-Thoneick et al. [77]. The heat production from oxygen intake (20.46 kJ l^{-1}) was calculated by indirect calorimetry. Because of a rise in energy requirements, the energy balance of the animals was negative under high ambient temperature.

The self-heating process of microorganisms in manure or hay has already been mentioned under the sections on animals and populations.

In the field of medical calorimetry, two publications on energy metabolism and entropy flow in man should be mentioned here. Using a suit calorimeter, Webb et al. [78] refuted the traditional assumption that level walking was only a heat evolving process with no work being done. Vertical and horizontal loads increased the fuel cost and heat loss of walking but did not alter the power output. The most likely explanation of the work done was in the interaction between feet and ground.

A positive entropy production in the human body was calculated by Aoki [79]. Independent of the persons tested and temperatures chosen (26–32 °C), the magnitude of this entropy production was nearly constant (0.172 J m^{-2} s^{-1} K^{-1}). Forced air around the human body and also clothing had almost no effect on entropy production, which does not seem to depend on environmental factors.

Nutrition

The influence of quality and quantity of nutrition (energy and carbon sources) on the calorimetrically measured heat production of animals and microorganisms in an ecosystemic context is the topic of this section.

The accurate estimation of the amount of energy in food measured by bomb calorimetry does not record the usable energy. Subtracting insoluble and indigestible compounds gave a more realistic but, nevertheless, not a precise value. The true usable energy of the prey was variable, and

depended on the physiological state of the predator. Thus, McClintock [80] pointed out that the guts of animals are not bomb calorimeters.

Feeding experiments were carried out by Lambert [81] to estimate the net caloric intake of the mollusc *Lamellaria diegoensis* on a colonial ascidian (*Cystodytes lobatus*) (4.7 cal g^{-1} d^{-1}; 19.7 J g^{-1} d^{-1}). The loss of energy in faeces was subtracted.

The fermentable part of organic matter, calculated from the microbial heat data of grass forage *in vitro* fermentation and the determined organic matter disappearing from dacron bags in the rumen of cows, were used by Arieli and Werner [82] to estimate the rate of energy digestion of fodder in the rumen.

Calorimetric investigations on human nutrition have not yet usually been connected with ecosystemic examinations. Nevertheless, microcalorimetric control of the microbial heat generated in ripened cheese [83] and in spoiling fruits [84], as well as the relation of microbial activity to the water content of food [85], should be mentioned here.

The earlier discussed identification of bacteria from microcalorimetric power–time traces [86] has been disputed by Perry et al. [87] and Bunker and James [88]. They pointed out that the choice and concentration of nutrients, the level of aeration, inoculum density, organism background and other cultural conditions, rather than the organism species, determined the nature of the trace.

The use of microbial heat production for detection of many compounds, e.g. acetate, methanol, sugars, etc., by adapted bacteria has been suggested by Lovrien et al. [89].

Degrading haloaliphatic compounds as the sole carbon source, the bacterium *Xanthobacter autotrophicus* was measured on-line by flow microcalorimetry and off-line by different biomass indicators. Greer et al. [90] indicated the possible elimination of some xenobiotics using such microorganisms.

Toxicants

As a new development in the last ten years, the detection of environmental pollutants by bioindicators has been systematically elaborated. Microcalorimetry was used preferentially for testing toxic chemicals such as heavy metals [91,92], 2-nitrophenol [93], pentachlorophenol [94,95], as well as platinum group metal complexes [96], an anti-slime agent [97] and antibiotics (ref. 98 and this volume). The test substances were usually added during the logarithmic growth phase of pure microbial cultures in synthetic culture media [98,99] or relative to non-growing organisms. Microorganisms were often investigated [93] but snails [92], earthworms, isopodes, or pill bugs [94,95] were also investigated, see also below. Some authors attempted to obtain a closer relation to natural conditions by

leaving the bioindicators in their ecosystem; for instance, measurements of heat evolution during the microbial degradation of glucose in soil in the presence of Hg, Cd, Se and iodoacetic acid as pollutants, of microbial heat production in litter and soil after incorporation of PCP [94,95] and of released energy as an indicator of microbial activity in papermaking water plus slimicide to differentiate between bacteriocidal and bacteriostatic effects [97]. A substantial advantage of microcalorimetry as compared to traditional bioassays is the continuous monitoring of the effect, the quick and simple performance [98] (a few hours instead of days) and the possibility of registering sub-lethal responses and unexpected reactions.

The rapid response of calorimetry, being a non-specific method for quantifying the enthalpy changes during metabolism, allows the kinetic reaction of the system and the variations of metabolic activities without any specifity to be monitored [31,93]. A further advantage of this method is its applicability in aerobic, anaerobic and mixed systems. The extent of fermentation versus respiration can be specified [100]. In contrast to photometric methods, sludge, soil, litter and waste water samples can be investigated directly because no optically clear solution is required [30]. The biological active biomass can also be estimated [16].

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