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APPLICATION OF THERMOANALYTICAL METHODS IN BIOCHEMISTRY AND BIOTECHNOLOGY

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ABSTRACT

Some new applications of isothermal and adiabatic calorimetry and differential scanning calorimetry (DSC) in biochemistry/biology and biotechnology are presented. Those processes which are connected with the microbial metabolism, its control and stimulation or hindering by drugs seem to be the most promising fields for further calorimetric investigations.

INTRODUCTION

Although biology stood at the cradle of calorimetry two hundred years ago, only recently calorimeters found a broader application in biology, medicine and related fields. No longer highly sophisticated instruments for specialists only, but sensitive set-ups comparable with better spectrophotometers a broader distribution of this method should be expected.

Talking about biological calorimetry a twofold division must be performed; that between isothermal and non-isothermal conditions and that between batch and flow instruments. In batch calorimeters the probe under investigation - let it be a biochemical system, a microbial culture or a small animal - is completely isolated from the environment facilitating the measurement of the heat production rate without disturbances. In flow calorimeters a microbial culture or a reacting system is pumped from an external fermentor through a heat exchanger in the calorimeter back to the fermentor. In such a set-up many different parameters of the culture can be determined simultaneously without interfering with the heat measurement: optical density, biomass, concentration of substrates and products, oxygen tension, pH,...

The second differentiation goes for isothermal and non-isothermal instruments. Most biological and biochemical experiments are run in the isothermal mode with isothermal-isoperibolic in-

struments. Only few investigations were done until now with adiabatic set-ups where the biologic heat production leads to a significant increase of temperature: decomposition or rottening of litter, compost or hay and self-heating processes in palm kernels, wool, jute and other products (see f.i. 1,2).

In contrast to these experiments with an "intrinsic temperature program" there is the broad field of - mainly biochemical - investigations with an external temperature program: DSC, DTA, DTG and further analytical methods (3).

Since the beginning of this century "indirect calorimetry" plaid an important role in biology: the determination of oxygen consumption and carbon dioxid production by an organism and the calculation of the heat production for known heat contents of the metabolic substrates. Only recently more attention was paid to differences which may appear between "direct" and "indirect" calorimetry on the same object (see below).

Biochemical and biological calorimetry was described in several monographs and in a number of review articles. Only a few shall be cited here (3-7).

ISOTHERMAL CALORIMETRY

Besides the division made above calorimeters in biochemistry and biology may be used as

- analytical tools just to detect a heat production and to monitore it without regard as to the quantity of heat which is evolved in the process under investigation (5). This form is used for identification/characterization of microorganisms (8-11), proof of contaminations in dairy products or sewage water (12,13) and testing of antislime agents on water quality in paper plants (14),
- quantitative tools to measure heat production and related thermodynamic figures in the classical sense. This includes the whole field of biochemical thermodynamics as well as metabolic investigations of microorganisms, tissues, organs or animals (15).

Identification/Characterization of microorganisms

In recent years a special field of analytical calorimetry grew up to some importance: the characterization or identification of microorganisms by their "finger-print" like power-time curves

during metabolism and growth. This field of research started 1973 (8) and was applied in clinical and biotechnical microbiology with varying success (9-11). Microorganisms growing in a complex medium exhibit a consecutive series of varying metabolic activities which are reflected in the rate of heat production. As the metabolic pathways differ in different microbes, structured power-time curves appear which are specific for the organism. But as a change in the composition of the medium strongly influences the metabolism too only strictly defined media can be applied (10).

In many cases an identification can be given within a few hours, much quicker than with conventional methods. But the drawbacks of this method are that it is not possible to work with mixed cultures or to run many probes at the same time as routinely with Petri-dishes. Multi-channel calorimeters would be a great help for this end.

Sometimes it is not necessary to identify microorganisms but only to control existing strains periodically for correct performances in dairy plants, bakeries or breweries (15). In these cases, simple well defined media composed from several sugars can be applied.

Testing of drugs

A field of interest for microbiology, medicine and pharmacy is the calorimetrically testing of the action of drugs on microorganisms. The identity: life = growth is no longer valid if we are not only looking for mere killing but for metabolic signals of an organism. As calorimetry is monitoring heat production and thus metabolism it renders more information as growth on Petri-dishes. Adding a drug to a growing culture of microbes and observing a constant heat production thereafter demonstrates that growth is inhibited while metabolism occurs in cells still alive: a bacteriostatic action of the drug. If the heat production decreases a bacteriocidic action is obtained. Again, such tests are by far quicker and sometimes even more sensitive than normal routine procedures (17,18).

Monitoring fermentative processes

Heat is the signal which is produced spontaneously by a living system and which is used to follow life processes without interference as in biochemical tests. The specific heat production is

modest calculated per gramm of wet weight, ranging from a few milliwatts for a higher animal up to several hundred milliwatts for microbes. These rates are really small and sensitive instruments are necessary to detect them, but if one deals with large fermentor units of some 10.000 liters the factor "heat production" becomes important. As microorganisms show their optimal growth or activity only in a narrow temperature range a severe control of temperature is inavoidable.

"Dynamical calorimetry" (19) determines the temperature difference between the in- and out-flow of cooling water and establishes a heat balance for the fermentor system taking into account further sources and sinks of heat. Flow-calorimeters measure heat production in a by-pass to the large fermentor (20). More interesting are specially designed submergable calorimeters (21) which are in total incorporated into the fermentor and determine there the heat production (not the temperature!). A further development might be the "psiumeter" discussed below.

Psiumetry

When one passes through the literature looking for simultaneous or parallel measurements of heat production and oxygen consumption in biologic systems, one frequently observes a more or less distinct difference between the heats calculated from oxygen consumption (measured manometrically or polarographically) and those determined directly by calorimetry (22). Zotin (23,24) was the first to construct a theory on this difference which is strongly connected with the dissipation of energy (psi-function). The "psiu-function" is that part of the dissipation function which is not dissipated sensu strictu to the environment but stored in the system. According to the thermodynamics of irreversible processes this difference should decrease during the development of a system and attain a final stationary value. Examples for such a behaviour may be found in (22).

As the psiu-function takes into account not only heat production or gaseous metabolism or biomass production but combines these three figures in one - mass specific - value of metabolism it seems to be the most appropriate method to evaluate biologic activities. Special interest should be paid here to monitore processes as fermentations and biomass production (25), single cell protein production or waste water treatment. Construction or deve-

lopment of special "psiumeters" is underway in several laboratories.

ADIABATIC CALORIMETRY

It was mentioned above that adiabatic calorimetry is hardly used in biologic research. This is understandable because most processes in organisms proceed under isothermal conditions. But there are several systems for which increasing temperatures under adiabatic or quasi-adiabatic conditions are typical (1,2). When larger amounts of organic material are piled up, such situations become observable: with straw, manure, hay, litter, compost and brushwood, but as well in bags of wool, corn, coffee or palm kernels. In some of these cases the danger of self-ignition exists.

The self-heating processes are a consequence of fermentative activities of mesophilic (up to 45°C), thermotolerant (up to 55°C) and thermophilic (up to 75°C) organisms followed by pyrogenic chemical processes. For pure chemical reactions such behaviour is followed by "Accelerating rate calorimetry" (26) or observed through exothermic effects in DSC-thermograms, but biologic material was mainly neglected. Besides the hazards connected with self-ignition the utilization of otherwise wasted energy becomes interesting in future (27). The amounts of energy stored in manure without burning it are considerable (2) and might be used on a small scale for single farms or in a larger scale with litter and compost for urbanic units. Calorimetry might find new ways of effective utilization of such energy sources.

DIFFERENTIAL SCANNING CALORIMETRY

Biological applications

Besides a few investigations about freezing injuries to buds of plants the main biologic field of interest for DSC are natural and synthetic membranes and their constituents. Many experiments are performed on vesicles, liposomes and complete membranes looking for thermotropic phase transitions. DSC is of great advantage in this field - as it is not necessary to introduce any external markers as with other methods - and increased our knowledge about membranes and their actions considerably (3).

Biochemical applications

Biochemical DSC investigations are mainly concerned with basic research on biologically relevant molecules such as carbohydrates, amino acids, proteins, lipids and nucleic acids. Besides that the different states of water in cells or model systems are important as well for whole cells as for membranes or protein complexes. It was observed that up to three different types of water might be present in cells: water bound to protein molecules, weakly retained by protein molecules (both types freezing at very low temperatures) and free water (28).

Binding of drugs to nucleic acids (DNA) is of great importance in medicine because it offers the chance of effective treatment of diseases. Besides the more global investigations with isothermal calorimetry (see above) specific binding experiments on artificial and natural DNA (29) help to develop better medicaments and to elucidate their way of acting.

A survey of biological and biochemical applications of DSC may be found in (3).

Biotechnological applications

DSC together with other methods like mass spectrometry, electrophoresis and gas chromatography is frequently applied in food technology

- to determine the constitution of food stuffs in a "fingerprint" like manner,
- to look for alterations which appear during thermal treatment (carbonizating, roasting, frying)
- to find strong exothermic reactions and to establish safe large scale industrial trials.

Examples of such DSC applications are investigations of cereals (30) with strong exothermic effects, analysis of meals and food grain legumes (31), coffee and chicory (32), cacao butter (33) or animal body fats (34), meat proteins (35) and fish meat gels (36) and of aged whiskey (37).

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