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What does calorimetry and thermodynamics of living cells tell us?

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ABSTRACT

This article presents and compares several thermodynamic methods for the quantitative interpretation of data from calorimetric measurements. Heat generation and absorption are universal features of microbial growth and product formation as well as of cell cultures from animals, plants and insects. The heat production rate reflects metabolic changes in real time and is measurable on-line. The detection limit of commercially available calorimetric instruments can be low enough to measure the heat of 100,000 aerobically growing bacteria or of 100 myocardial cells. Heat can be monitored in reaction vessels ranging from a few nanoliters up to many cubic meters. Most important the heat flux measurement does not interfere with the biological process under investigation. The practical advantages of calorimetry include the waiver of labeling and reactants. It is further possible to assemble the thermal transducer in a protected way that reduces aging and thereby signal drifts. Calorimetry works with optically opaque solutions.

All of these advantages make calorimetry an interesting method for many applications in medicine, environmental sciences, ecology, biochemistry and biotechnology, just to mention a few. However, in many cases the heat signal is merely used to monitor biological processes but only rarely to quantitatively interpret the data. Therefore, a significant proportion of the information potential of calorimetry remains unutilized. To fill this information gap and to motivate the reader using the full information potential of calorimetry, various methods for quantitative data interpretations are presented, evaluated and compared with each other. Possible errors of interpretation and limitations of quantitative data analysis are also discussed.

1. Introduction

Heat is an inevitable by-product of any microbial growth and product formation processes. Heat is directly related to growth stoichiometry and heat production rate to the metabolic fluxes via Law of Hess. Calorimetry measures the metabolic activity of a population of cells. By this, any metabolic change can be monitored on-line and in real time. These days, heat production rate can be measured with a specific limit of detection of 10^{-5} W L^{-1} which corresponds to a microbial oxygen depletion of air saturated medium (approx. 6.7 mg L^{-1} at 37 °C) within 44 days. In case of aerobic glucose combustion it relates to the tiny consumption rate of 0.03 μM h^{-1} or 5 μg L^{-1} h^{-1} [1]. These data show the potential of calorimetry. A good overview about the dependency of the limit of detection on the considered reaction volume and on the applied instrument is given by Zogg et al. [2]. Calorimetry works in opaque media and doesn't need any labeling agents. Long-lasting and elaborated optimization and design of primer, probes and hybridization conditions as known for molecular biology-based methods [3] are not required in calorimetry. Finally, thermal transducers may be, different to many other biochemical sensors, mounted in a protected location. This keeps the transducer stable for long time and reduces the signal drift. All of these advantages increase the scientific and technical demand for calorimetric measurement methods and data interpretation. As a result commercially available highly sensitive, high throughput calorimeters, able to measure up to 48 channels in parallel have been developed. Fields of application ranges from medical research [4], environmental microbiology [5–7] to the food industry [8]. Due to the development of highly sensitive calorimeters with a high throughput and their validation in different application fields the quantitative data interpretation becomes more and more important. First reports on enthalpy balances around systems of different scales which are also applicable to calorimeters, bioreactors or even ecosystems were published more than two decades ago [9,10]. The authors correlated metabolic fluxes and growth stoichiometry with heat flows via Law of Hess. However, advanced chemical analytics are indispensable for such a data analysis. Quite often comprehensive

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metabolic information are not available for interpretation of calorimetric experiments. For this, the applicability of more simple growth models assuming a constant heat production rate per cell or per converted electron were successfully tested just recently [11]. Finally, the recent tendency to simplify and to standardize calorimetric measurements leads to potential misinterpretations of calorimetric experiments [12,13].

This review relates different types of calorimetric data interpretation to the level of available information. It explains weaknesses and advantages of the evaluation methods. Also, causes of various misinterpretations of calorimetric measurements are going to be discussed. Finally, the compromise between modern trends in respect to miniaturization and high-throughput measurements and the requirement for more analytical information for a correct calorimetric data interpretation will be discussed.

2. Extracting quantitative information from the calorimetric signal

2.1. Data interpretations using the first law of thermodynamics

Every open system, independent of its size and the ability to monitor the heat production, can be considered as a type of calorimeter. A "system" can be a part of an ecosystem, a bioreactor, an animal or a plant but it could also be a simple medical or food sample containing active cells. Clearly defining the systems boundaries is essential for designing the experiments and later for data evaluation. Fig. 1 shows a simplified chart of such an open system exchanging energy and matter with the environment.

The general balance of such a system has the structure of:

$$\text{Accumulation in the system} = \text{Input} - \text{Output} - \text{Chemical, biological conversions} \quad (1)$$

Every conserved quantity (e.g., elements, electrons, individual chemical and biological species as well as enthalpy) can be balanced. The usage of enthalpy balances for evaluation of calorimetric results makes most sense if information about the metabolic fluxes r_i (Eq. (2)) or about the growth stoichiometric coefficients $Y_{i/X}$ (Eq. (3)) is available or aspired. The difference between heat and enthalpy is discussed in a later section of the article. However, the main barrier to application of fully balanced systems is the requirement of an exhaustive chemical analysis of the calorimetrically monitored process. The following example shows the simplest case of aerobic biomass formation ($CH_{X1}O_{X2}N_{X3}$) from any organic carbon source ($CH_{S1}O_{S2}$). The cell dry mass of biological

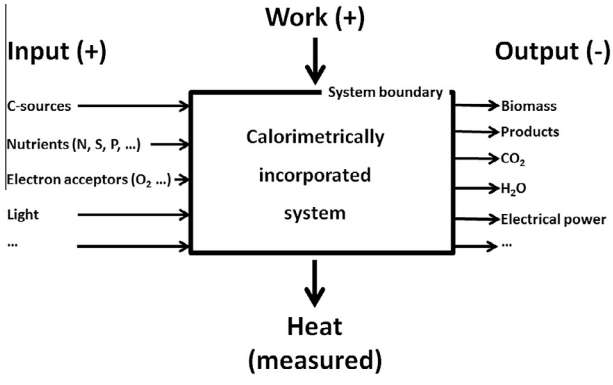

Fig. 1. Calorimeter as an open system exchanging energy and matter with the environment. The calorimeter can incorporate parts of ecosystems, bioreactors or simple samples with living matter. The system can be in steady state or in a transient. It has to be adapted to the specific experimental conditions. Convective heat flows due to exchange of matter is not considered. The figure is derived from [9].

organisms is designated in the following as biomass. The elemental composition of X1 = 1.70; X2 = 0.42 and X3 = 0.25 is typical for bacterial biomass [14].

$$r_S\ CH_{S1}O_{S2} + r_N\ NH_3 + r_{O_2}\ O_2 \rightarrow r_X\ CH_{X1}O_{X2}N_{X3} + r_{CO_2}\ CO_2 + r_{H_2O}\ H_2O \quad (2)$$

with $Y_{i/X} = \frac{r_i}{r_X}$

$$\text{follows}\quad Y_{S/X}\ CH_{S1}O_{S2} + Y_{N/X}\ NH_3 + Y_{O_2/X}\ O_2 \rightarrow CH_{X1}O_{X2}N_{X3} + Y_{CO_2/X}CO_2 + Y_{H_2O/X}H_2O \quad (3)$$

The five unknown stoichiometric/yield coefficients $Y_{i/X}$ are not independent of each other. They have to fulfill four elemental balances for C, H, O and N. This means the yield coefficients can be calculated if one more conditional relation is known. This additional relation can be the enthalpy balance which is also called the Law of Hess (Eq. (4)).

$$\Delta_R H_X = \sum_{i=1}^{n}(Y_{i/X}\,\Delta_f H_i) = -\sum_{i=1}^{n}(Y_{i/X}\,\Delta_C H_i) \quad (4)$$

The energy balance/Law of Hess correlates the measured growth reaction enthalpy $\Delta_R H_X$ with the energy contributions of each chemical species i involved in the growth reaction. $\Delta_R H_X$ is the enthalpy released (negative sign) or consumed (positive sign) during the formation of one mole biomass. The energy contribution of each species can be described as enthalpies of formation $\Delta_f H_i$ or of combustion $\Delta_C H_i$ (see Eq. (4)). However, the correct standard and reference state of the enthalpy of all species have to be selected and to be corrected for temperature. Von Stockar and co-workers discussed consequences of wrong usage of reference states and neglecting temperature corrections [10]. Note, the usage of combustion enthalpies simplify the enthalpy calculation (in this specific case) because the combustion enthalpies of CO_2 and H_2O are zero (see Eq. (5))

$$\Delta_R H_X = -(\Delta_C H_X - Y_{S/X}\,\Delta_C H_S - Y_{N/X}\,\Delta_C H_N) \quad (5)$$

The calorimetrically determined heat production rate $\dot{Q}$ contains additional kinetic information[15,16]. Eq. (6) shows it at the example of the growth rate r_X.

$$\dot{Q} = r_X\,\Delta_R H_X \quad (6)$$

The growth reaction heat ($\Delta_R Q = \int \dot{Q}\,dt$) is equal to the growth reaction enthalpy $\Delta_R H$ for open systems with a constant pressure ($dp = 0$). In case of calorimetric measurements in the often used closed ampoules, a contribution of work ($\int V\,dp$) has to be added. In case of a typical ampoule of 4 mL with 2 mL cellular suspension and 2 mL air space a pressure increase of 1 atm (101,325 Pa) provides only 0.2 J, which can often be neglected in comparison to the growth heat.

Calorimetry measure the average metabolic activity of a population of cells. An in-depth data interpretation can only be done if enough analytical information is available. The calorimetric systems must be large enough for extensive sampling or to include online sensors. This is the case for experiments in a reaction calorimeter, in bioreactors combined with a flow through calorimeter [17–20], or by performing a calorimetric experiment in parallel to an equivalent experiment in a bioreactor. The latter two are error-prone due to metabolic processes in the flow through line or wall growth [21,22]. Differences between the physical environment in the calorimeter and in the bioreactor may distort the calorimetric result. An experiment must be carefully designed to minimize distortions of the calorimetric signal due to (i) the addition of substances with deviating temperatures, (ii) the heat of neutralization by keeping the pH constant, (iii) heat of evaporation due to aeration, (iv) heat effects of gas adsorption and release, and