Methods 76 (2015) 27–34

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Microcalorimetric assays for measuring cell growth and metabolic activity: Methodology and applications

O. Braissant [a,b,*], A. Bachmann [a], G. Bonkat [a,b]

[a] Department of Urology, University Hospital Basel, Basel, Switzerland
[b] Laboratory of Biomechanics and Biocalorimetry (LOB2), University of Basel, Basel, Switzerland

ARTICLE INFO

Article history:
Received 29 August 2014
Received in revised form 7 October 2014
Accepted 8 October 2014
Available online 15 October 2014

Keywords:
Isothermal microcalorimetry
Bacteria
Fungi
Analytical method
Microbial growth
Microbial activity

ABSTRACT

Isothermal microcalorimetry measures the heat released or consumed by physical or chemical processes. Metabolic activity releases heat that can be measured. However the calorimetric signal can be difficult for new users to interpret. This paper compares microcalorimetry to other techniques and reviews its application to different fields where microbiological activity is important. We also describe different ways to analyze the data and translate it into meaningful (micro)biological equivalents. This paper aims at providing non-specialist reader the tools to understand how isothermal microcalorimetry can be used for microbiological applications.

1. Introduction

1.1. General

Isothermal microcalorimetry is a very sensitive technique to monitor microbial metabolic activities and growth. With some current instruments, only 10^4 active microbial cells or 1000 rat hepatocytes can release enough heat to be detected [1–3]. This makes isothermal microcalorimetry rather appealing for such monitoring. However to understand what use can be made of this technique, it is important to compare it with other assays commonly used in microbiology, to be able to translate the calorimetric signal into a meaningful microbiological data and finally, to outline its strengths and weaknesses. One aim of this paper is to show how isothermal microcalorimetry can complement or in some cases outcompete other methods.

Monitoring growth of bacterial, fungal or mammalian cultures is crucial in many different settings such as drug development, toxicology studies, clinical investigation and environmental studies. In addition to research purposes, monitoring cell growth, and particularly microbial growth, is crucial in many industrial processes such as food production or biomedical applications [4,5]. In many cases, monitoring the increase of the cell population is sufficient to assess the effect of a defined parameter (the efficacy of a new drug, or the effect of a toxic compound for example [6]).

Measuring the metabolic activity (or a specific enzymatic activity) of cells is also of great importance. In several contexts, one might encounter live organisms in a metabolically inactive state. Such organisms include dormant or resting cells. On the contrary, viable but non-culturable cells [7,8] are also commonly encountered. In soils for example it is considered that only 1–10% of cells can be grown on appropriate medium [7]. Similarly, in urine or in the gut only a fraction of the organisms present can be isolated and cultivated [9,10]. Therefore using cell counts only might lead to large over or underestimation of a process, and measurement of specific activities is often desirable.

1.2. Non calorimetric techniques (an overview)

With respect to monitoring prokaryotic or eukaryotic cell growth, in many cases the conventional culture and plate counts on appropriate medium remains considered as the gold standard. However plate counts are time consuming and deliver results only after 24–48 h in most cases. For some fastidious organisms such as *Mycobacterium tuberculosis* this can take up to several weeks [11]. In this context many alternative methods have been used to monitor microbial growth [12,13]. Among those, it is not surprising that

* Corresponding author at: Center for Biomechanics and Biocalorimetry (COB2), Faculty of Medicine, University of Basel, c/o Biozentrum-Pharmazentrum, Klingelbergstrasse 50-70, 4056 Basel, Switzerland.
E-mail address: Olivier.braissant@unibas.ch (O. Braissant).

http://dx.doi.org/10.1016/j.ymeth.2014.10.009
1046-2023/

optical methods such as spectrophotometry and microscopy are widely used to assess the growth of cell culture [6]. In addition, biochemical assays allowing the quantification of a cell component such as protein [14], sugar [15], DNA [16] or ATP [17] are also often used to estimate the number of cells in a culture. Finally in some context, measuring the impedance has been shown to be an accurate method to estimate cell number [13,18–20].

To monitor the metabolic activity of cells or microbial cultures, researchers often use so-called metabolic assays based on the reduction of tetrazolium dyes or resazurin by dehydrogenase mostly from the respiratory chain [21]. Monitoring oxygen consumption rate or byproduct appearance, such as H_2S for sulfate reducing bacteria, ion specific electrodes can be used as well [22]. Also, to assess respiration rates, fluorescein diacetate has been used. Other assays can target specific enzymatic activities such as glucosidase, glucanase, glucosaminidase [23,24], sulfatase [25] or phosphatase [26] using chromogenic or fluorogenic substrates.

1.3. Isothermal microcalorimetry

In this context, isothermal microcalorimetry is an appealing tool for several reasons that will be detailed hereafter. Isothermal microcalorimetry measures the amount of heat released by any physical or chemical process [27]. In the present case the heat released by the many biochemical reactions taking place in a metabolically active cell. The total heat released over time is proportional to the advancement of the reaction considered. Considering a simple model (see Eq. (1)) one can see that the amount of heat produced is theoretically proportional to the amount of substrates consumed, the biomass produced and the byproduct released [28–30]:

$$\text{Substrate}_1 + \ldots \text{Substrate}_n \rightarrow \text{Biomass} + \text{byproduct}_1 + \ldots \text{byproduct}_m + \text{heat} \quad (1)$$

The substrates are the medium component (i.e., mostly carbon and nitrogen source) and byproducts are the product of the metabolism. This is of course a very simplified model to illustrate how a microbiologist can interpret the overall heat release. Much more detailed models to explain the heat released have been proposed [31–34]. Also prokaryotic and eukaryotic cells are known to engage in futile cycles for several reasons [35,36]. In such case such models should be taken with care since no biomass would be produced. Also the heat production per unit time (i.e., the heat flow or thermal power) that is the derivative of the total heat over time curve is directly proportional to the speed at which the considered reaction takes place and can be considered as a proxy of the metabolic activity. Therefore one often finds that the thermal power expressed in Watts (Joules seconds^{-1}) can be correlated with metabolic assays such as tetrazolium salt or resazurin reduction [37–39]. Similarly heat can be correlated to assays measuring the amount of biomass produced (DNA, protein, dry weight), the oxygen consumed or metabolic byproduct released (NO_2 for denitrifying bacteria or H_2S for sulfate reducing bacteria [40,41] Fig. 1). With respect to respiration and oxygen consumption, the Thornton rule [42] might be useful. Indeed the enthalpy of combustion is directly proportional to the amount of oxygen consumed (or to the amount of electron required to reduce O_2 into H_2O). In this context it is commonly admitted that 455 kJ are liberated for the consumption of 1 mol of O_2 [43,44]; this value is referred to as the oxycaloric equivalent. In closed calorimetric vials the oxygen amount in solution can be measured using oxygen electrodes or approximated using the Weiss equation [45] and thus the heat generated by respiration can be approximated as well [1,46]. As many microorganisms prefer using O_2 as terminal electron acceptor, the first peak encountered in thermogram is often linked to respiration and the area under the curve (i.e., the integral) of this peak usually fits with the calculation based on the Thornton rule combined with the amount of dissolved oxygen (Fig. 1).

Isothermal microcalorimetry becomes even more appealing when it is compared to the other commonly used techniques and especially when the limitation of each technique is taken into account. Table 1 provides a summary of the sensitivity and applicability of those techniques. The sensitivity of isothermal microcalorimetry makes it an appealing tool. In addition, because isothermal microcalorimetry is label free and does not require a liquid or transparent sample it becomes obvious that it is particularly well suited for measuring growth in solid or opaque samples [47]. Finally, although one needs to have ca 10^4–10^5 cells to reach the detection limit of most instruments, most cells (and especially microbes) grow fast and such number can be reached rapidly. Thus in theory, and given enough time, one microbe is enough for detection of growth.

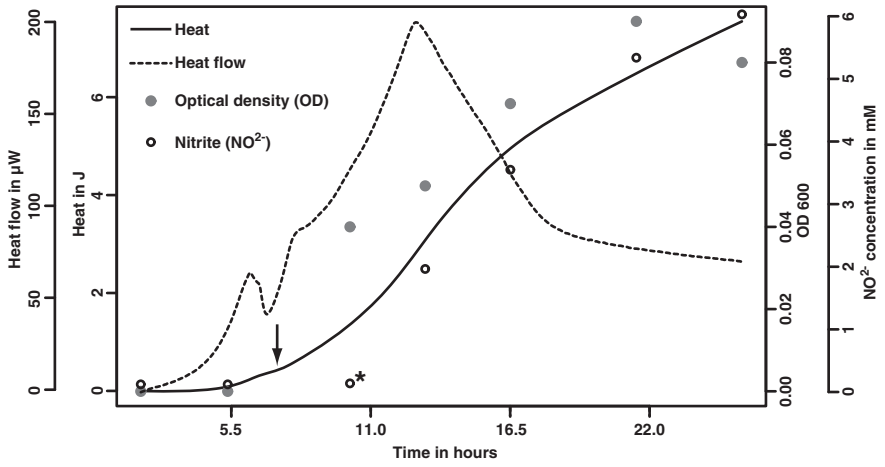

Fig. 1. Thermogram collected during the growth of *P. aeruginosa* in BME medium added with 100 mM of potassium nitrate (Braissant unpublished data). Note that the heat correlates well with the optical density (i.e., biomass) and nitrite (i.e., resulting from nitrate reduction) increase. The arrow indicates approximately the time point where the oxygen is depleted in the medium. In this case the first metabolic peak is considered as respiration (using oxygen), the heat released at this time point correspond roughly to the heat released by the respiration of organic matter using an amount of oxygen determined using the Weiss equation (see text for details). After deconvolution of the first peak we estimated that 356 ± 18 mJ (n = 10) of heat were released when oxygen was exhausted. Using the Weiss equation we estimated the amount of oxygen in the 3 ml vial to 0.8 µmol. With this amount of oxygen the Thornton rule predicts a heat production of 364 mJ. Thus fitting quite well with our observation that the first peak can be attributed to O_2 respiration. This is further supported by the fact that denitrification only takes place in anoxygenic conditions.