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A method for time-resolved calorespirometry of terrestrial samples

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ABSTRACT

A new vessel for simultaneous isothermal calorimetry and respirometry (calorespirometry) on terrestrial (non-aqueous) samples has been developed. All types of small (<1 g) biological samples (insects, soil, leaves, fungi, etc.) can be studied. The respirometric measurements are made by opening and closing a valve to a vial inside the sample ampoule containing a carbon dioxide absorbent. Typically a 7 h measurement results in seven measurements of heat production rate, oxygen consumption and carbon dioxide production, which can be used to evaluate how the metabolic activity in a sample changes over time. Results from three experiments on leaves, a cut vegetable, and mold are given. As uncertainties – especially in the carbon dioxide production – tend to be quite high, improvements to the technique are also discussed.

1. Introduction

Calorespirometry (CR) is the simultaneous determination of thermal power (heat production rate) and oxygen consumption rate and/or carbon dioxide production rate. This can be a powerful technique to make an overall characterization of biological systems [1,2]. Calorespirometry has mainly been used to study biological systems, see for example Refs. [2–5], but can also be used for non-biological systems [6,7].

There are different ways to perform CR measurements. In this paper a further development of the different CR techniques used by Hansen and co-workers for terrestrial samples, i.e., samples in the gas phase, is presented. In their most common experimental setup (see for example Refs. [2,3,8–12]) the heat production rate (thermal power) is measured in an isothermal calorimeter, while the carbon dioxide production is determined from the difference between the thermal power measured for the sample only, and for the sample with a carbon dioxide absorbent. As the reaction between carbon dioxide and the absorbent has a known enthalpy, the carbon dioxide production rate can be calculated from the difference in thermal power with and without the absorbent.

In some variants of CR on terrestrial samples the calorimetric measurements are complemented with pressure measurements. From the pressure change rate with the absorbent the oxygen consumption rate can be calculated by the ideal gas law. The ratio between heat production rate and oxygen consumption rate can then be compared with the almost constant enthalpy of combustion of organic compounds (Thornton's constant [13]) of about −455 kJ mol^{-1} (O_2) [14]. A measured value higher than Thornton's constant indicates that the process is partly anaerobic [15,16].

It is also possible to use oxygen and carbon dioxide sensors, but this has mainly been done in the aqueous phase as such sensors often do not perform as well in the gas phase. Sensors may also have other drawbacks – such as high heat production rate, large size and drift – that at present prevent their successful use in terrestrial calorespirometry.

A potential problem with the absorbent method (as described above) is that the sample ampoule has to be taken out of the calorimeter and opened to put in the absorbent vial. This may cause disturbances to the respiration, and as that operation is usually made twice (commonly a measurement is made without–with–without the absorbent [17]) it may be difficult to assess whether the differences seen between the two modes are caused by the sample or by other effects. It is also my experience that the activity of biological samples in a calorimeter seldom is constant. Whole photosynthetic tissue normally shows a decreasing thermal power, while damaged tissue may show an increasing activity from the wound healing processes. Constant activity can be seen in systems in which there are no substrate limitations.

The method described in this paper makes it possible to perform a series of measurements with–without the carbon dioxide absorbent without disturbing the calorimetric measurement. This is made by having the absorbent vial with a valve fixed in the calorimetric ampoule. The valve can be opened and closed from outside the calorimeter. This time-resolved CR can be used to follow processes over time, and may also give more reliable values of the measured parameters as there are no large disturbances from taking out and putting back the ampoule in the calorimeter.

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The new CR ampoule also contains a pressure sensor to allow measurements of O_2 consumption rate.

2. Experimental

2.1. Method

The new CR vessel is shown in Fig. 1. The ampoule with the sample and the absorbent vial is made of stainless steel with a PEEK lid sealed by an o-ring. It is made to fit in a TAM Air calorimeter (TA Instruments, New Castle, DE, USA). The carbon dioxide absorbent vial is placed in the center of the ampoule. The absorbent vial has a 4 mm hole in the side through which carbon dioxide can pass into the absorbent when a valve (also made of PEEK) is positioned so that a hole in the valve corresponds to the hole in the vial. To close the absorbent vial the valve is turned 180°. The valve is manually operated from outside the calorimeter. The shaft on which the valve is placed passes through a stainless steel tube fixed in the lid of the ampoule. Between this tube and the shaft an o-ring is placed to make the ampoule pressure tight.

A small pressure sensor (Honeywell 26PC15SMT) is placed about 6 cm above the lid, so that it is placed in the temperature controlled air of the instrument. It is connected to the ampoule through a stainless steel tube (inner diameter 0.7 mm); the connection is made with a short rubber tubing that can be opened to prevent high/low pressures when the ampoule is being closed/opened. As the whole CR-vessel – including the pressure sensor – is placed in the temperature controlled environment of the instrument, the temperature of the pressure sensor is very stable. A three day run with empty ampoule gave a constant pressure reading within 0.5 Pa with a short term noise of ±1 Pa.

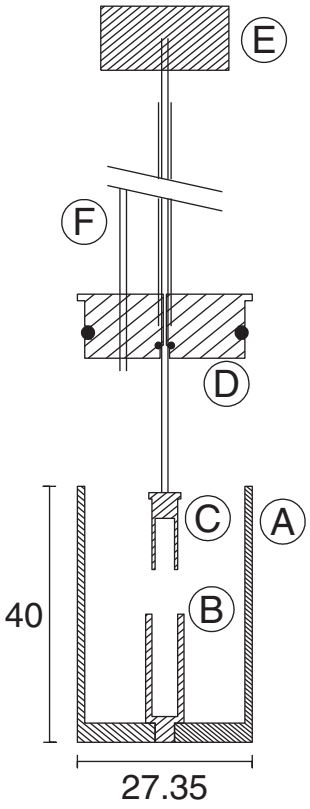

Fig. 1. The calorespirometric vessel shown in an un-assembled state (pressure sensor and plugs for TAM Air calorimeter are not shown; lengths in mm). (A) Ampoule in which the sample is placed. (B) Absorbent vial in which the hydroxide solution is placed. (C) Valve. (D) Lid through which the valve shaft and the tube to the pressure sensor passes. (E) Handle to open/close the valve. (F) Tube to pressure sensor.

The calorimetric vessel was calibrated electrically by placing a small heater and some paraffin oil in the ampoule. The resulting calibration coefficient is believed to be accurate to within about 1%. The baseline was measured with an empty ampoule: its value is believed to be correct within about 1% of the lowest heat production rates measured. All calorimetric results have been corrected for the thermal inertia of the calorimeter by the Tian equation [18]. The time constant was found by correcting measurements made with the vessel empty with different time constants, and choosing the time constant that gave the best correction (sharpest peaks from valve openings and valve closures, but no overshoot).

The pressure sensor was calibrated by connecting it to a glass bottle (volume 609.1 cm^3) to which a 60 mL syringe was connected. Steps in pressure were made by changing the volume in the syringe and the calibration coefficient was calculated as sensor output (voltage) change divided by calculated pressure change. The pressure change calibration coefficient is believed to be accurate to within about 6% (two standard deviations). The volume of the empty ampoule was calculated from caliper measurements and was 13.30 and 13.63 cm^3 for closed and open vial, respectively. In the evaluation, these volumes were adjusted for the sample volume.

The measurements were made in cycles in which the valve was open for about 4000 s and closed for about 500 s (see Section 4).

2.2. Materials

Measurements have been made on three types of biological samples. Shoots of white clover (*Trifolium repens*) were taken before mid-day after having been exposed to the sun for about 5 h. Cut carrot tissue was made by taking about 10 pieces of about 5 mm × 5 mm × 1 mm of the outer part of a carrot (*Daucus carota* cv. Nerac, commercial product). Samples of moist bread with mixed microbiological growth (mainly *Penicillium* mold) were cut from moldy bread. In all measurements the absorbent solution was NaOH (aq, 0.4 M) made from sodium hydroxide concentrate (Fixanal, Sigma–Aldrich).

2.3. Evaluation

The primary results of calorespirometric measurements with the described method are heat production rates and pressures. However, the results of interest are the heat production rates P (W) and pressure change rates Π (Pa s^{-1}) both when the absorbent vial is open (o) and when it is closed (c) (the nomenclature is given in Table 1). The measurements thus give four parameters as a function of time: P_o, P_c, Π_o and Π_c. These parameters can be further evaluated to give information on the metabolic processes in the biological samples.

The heat production rate when the vial is closed is the heat production rate of the sample, which is equal to the metabolic heat production rate, R_q

$$R_q = P_c. \tag{1}$$

If the enthalpy of the metabolic process is known the rate of the process can be calculated. For a fully aerobic process the oxygen consumption rate can be calculated using Thornton's constant that has a value of about −455 kJ mol^{-1} O_2 [14].

The difference between the heat production rate when the vial is open and closed is the heat production rate of the carbon dioxide absorbent. This can be recalculated to carbon dioxide production rate R_{CO2} (mol s^{-1}) as the enthalpy of the absorption process $\Delta_{abs}H$ (J mol^{-1}) is known

$$R_{CO2} = \frac{P_o - P_c}{\Delta_{abs}H}. \tag{2}$$