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# A gas perfusion microcalorimeter for studies of plant tissue ☆

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**Abstract**

A gas perfusion microcalorimetric system has been developed for metabolic investigations of plant tissues under dark conditions. The system consists of two twin heat conduction microcalorimeters connected by gas-tight tubing. During measurements, a flow of humid gas enters the main calorimeter containing the plant tissue. The volume of this sample chamber can be varied between 4 and 25 ml and the flow rate of the gas can be varied up to maximum of 150 ml $h^{-1}$. The outgoing air is allowed to pass through a second calorimeter, serving as an on-line $CO_2$ analyzer. Gases, such as $NO_x$ or $CO_2$, can be added to the incoming gas-flow either continuously or by stepwise injection. The calorimetric system has been tested in respiratory experiments with potato tuber slices.

*Keywords:* Gas perfusion microcalorimeter; Microcalorimetry; Potato

## 1. Introduction

The heat flow generated by living tissue is a general indicator of metabolic activity. Consequently, calorimetry provides an important, and now easy-to-use,

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analytical tool for noninvasive metabolic studies. While the technique has been extensively used to study micro-organisms and animal cells [1], comparatively few studies have focused on plant tissues, until recently (reviewed by Criddle et al. [2] and Hansen et al. [3]). Lately, reports have appeared using calorimetry to investigate the effects of stress factors such as salinity [4–6] and temperature [7–11]. Calorimetry has also been used to predict long-term growth rates of redwood [12–15], larch [16] and eucalyptus [9] trees from measurements of heat flow and respiratory gas exchange rates of small shoot tip segments ( $\leqslant 1$ cm). These predictions are made in the context of a recently published thermodynamic model relating growth rates to respiratory parameters [17].

In most of these studies, plant material is incubated in closed, non-stirred, calorimetric vessels during an experiment. Typically, the vessel contains a shoot, or a piece of a leaf, sometimes placed on filter paper soaked with medium. When experiments are done in closed vessels, the gas phase composition continuously changes due to tissue metabolism. While oxygen concentration will decline, normally it is adequate for several hours of incubation [18,19]. Of more concern is the accumulation of $CO_2$ and other volatile metabolic products, because they may inhibit or stimulate respiration, alter respiratory pathways, or otherwise affect the physiology and biochemistry of the tissue being measured [20–23].

Calorimetric vessels allowing a continuous flow of liquid or gaseous medium through the sample container have the advantage that the incubation conditions can be kept essentially constant or can be purposefully altered during extended measurements. Liquid or gas perfusion facilitates the introduction of additives, such as pollution gases or other metabolic affectors. Perfusion vessels also have the important advantage that they can be combined with an automated system for external analysis of the effluent gas or liquid composition relatively easily [24,25]. A complication with gas perfusion vessels arises from the need to control precisely the humidity of the incoming gas flow so that condensation or evaporation processes can be avoided in the calorimetric vessel.

Here we report a microcalorimetric perfusion system primarily designed for investigation of plant tissue incubated in a gaseous atmosphere, under dark conditions. The system consists of two heat conduction calorimeters connected in series by gas-tight tubing. The first calorimeter measures the metabolic heat flow, whereas the second calorimeter traps $CO_2$ in an alkaline solution [26,27] and measures the resulting heat of solution and neutralization, giving a continuous record of the $CO_2$ production rate of the plane tissue. The design of the calorimeters resembles our earlier modular microcalorimetric systems [28,29]. The performance of the system is demonstrated in respiratory experiments with potato slices where pronounced and well-characterized changes in the rate and character of respiration are known from other methods of investigation [30].

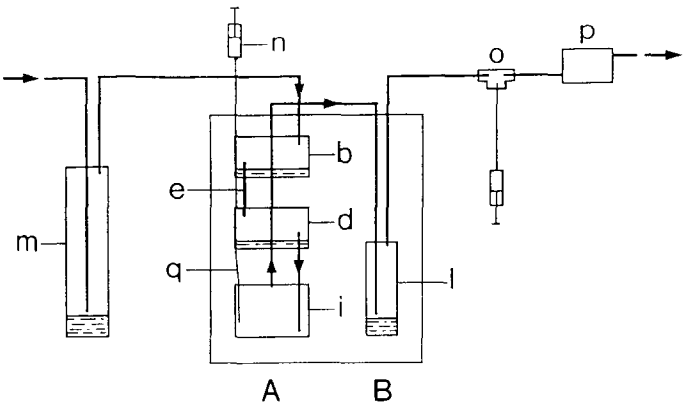


Fig. 1. Schematic diagram of the gas flow line of the calorimetric system: A, main calorimeter measuring heat flow from the plant tissue; B, calorimeter operating as a $CO_2$ analyzer; b and d, preequilibration cups; e, stainless steel tube; i, sample chamber; l, $CO_2$ neutralization chamber; m, $CO_2$ scrubber; n, injection syringe; o, gas sampling septum; p, peristaltic pump; q, injection needle.

## 2. Experimental

### 2.1. Instrument

Fig. 1 shows a schematic diagram describing the gas flow line of the calorimetric system. The two twin heat conduction calorimeters are shown in some detail in Fig. 2 (A and B). Both instruments are immersed in a thermostated water bath ( $\pm 0.1$ mK) [31]. The main calorimeter (A) [32] holds a removable 4.5 ml perfusion vessel (i) containing the plant tissue. A stainless steel tube (j, Fig. 2) connects the gas outlet from this calorimetric unit to the second calorimetric unit (B) [31], which holds a simple 5 ml gas perfusion vessel (l) charged with 1 ml of 1 M NaOH solution. Calorimeter B serves as an on-line $CO_2$ analyzer, based on the same principles as those used by Criddle et al. [26,27] in their closed vessel system.

Perfusion gas is drawn through the calorimetric system at a constant flow rate ( $\leqslant 150$ ml $h^{-1}$) by means of a peristaltic pump (p, Fig. 1) (Model A 1190, H.J. Guldener, Zürich, Switzerland). Before entering calorimeter A, the gas passes through a scrubber (m, Fig. 1) filled with 1 M aqueous NaOH, acting as a prehumidifier and $CO_2$ trap. The gas flow then passes through two brass preequilibration cups (b and d), connected by a stainless steel tube (e), before entering the stainless steel sample chamber (i). The equilibration cups are similar in design to those used in the thermometric vapor sorption vessel (Model No. 2255, Thermometric AB, Järfälla, Sweden) and in a recently reported vapor sorption calorimeter designed at this laboratory [32]. Each unit consists of a 7.5 ml brass cup filled with 1 ml of a solution with suitable vapor pressure. The cups are fitted with vertical inlet and outlet tubes for the gas flow. The lower cup (d) is in thermal contact with

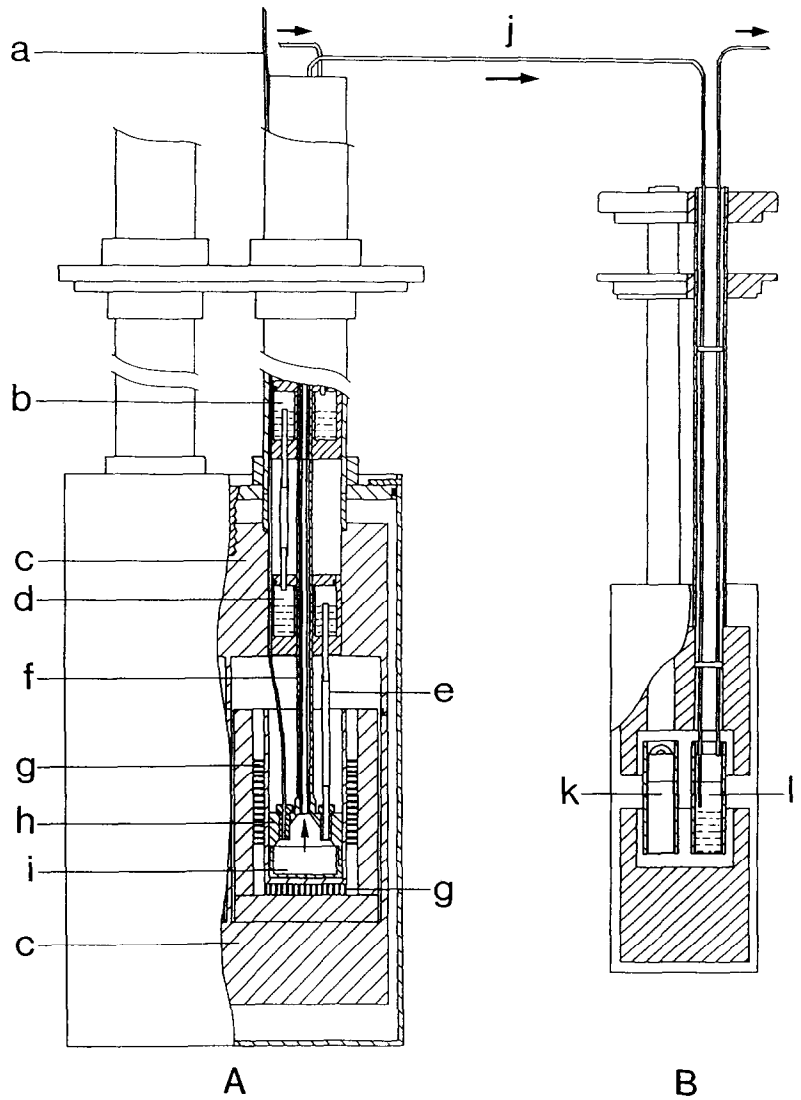


Fig. 2. Detailed diagram of the two calorimetric units: A, main calorimeter; B, $CO_2$ analyzer; a, stainless steel needle guide; b and d, brass preequilibration chambers; c, aluminum heat sink; e, stainless steel tube; f, central shaft carrying the outgoing gas flow; g, thermocouple plate; h, lid of the sample chamber; i, stainless steel sample chamber; j, stainless steel tube connecting the outgoing gas flow of calorimeter A to the $CO_2$ analyzer (B); k, reference vessel: l, stainless steel $CO_2$ neutralization chamber.

the aluminum heat sink (c, Fig. 2) whereas the upper cup (b) is in thermal contact with the thermostated bath. Gaseous reagents can be added to the gas flow as it enters the sample container (i), using a motor-driven syringe (n, Fig. 1) fitted with

a long hypodermic needle (q, Fig. 1). The needle is guided into the sample container by a thin steel tube (a, Fig. 2) (i.d. 0.5 mm). From the sample container the gas passes through a steel tube (j) to the 5 ml reaction vessel of calorimeter B (1) where the $CO_2$ produced by the respiration of the plant tissue will be absorbed by 1 ml of 1 M aqueous NaOH, giving rise to a heat flow proportional to the $CO_2$ flux. The septum (o, Fig. 1) provides a convenient way of sampling the effluent gas for further analysis.

### 2.2. Calorimetric experiments

The calorimeters were operated at a temperature of 25°C and at a gas flow rate of 45 ml $h^{-1}$, if not otherwise stated. In all experiments, the preequilibration cups of calorimeter A (b and d, see Figs. 1 and 2) were filled with 0.9% NaCl(aq) so as to match the vapor pressure of the incoming gas as closely as possible to the vapor pressure of the plant tissue. The time required for vapor pressure equilibration at the start of the experiments was reduced by covering part of the inner surface of the sample container of calorimeter A with a filter paper soaked in aqueous 0.9% NaCl.

Both calorimeters were thermally calibrated using a resistance heater mounted inside the respective vessels. The $CO_2$ analyzer (B) was calibrated for its response to $CO_2$ by injecting a known $N_2/CO_2$ gas mixture (Alfax, Sweden) into the main gas flow by means of the syringe (n, Fig. 1). The $N_2/CO_2$ gas mixture was injected continuously for approximately 1.5 h at each of the defined injection rates used for calibration.

Potatoes used in the metabolic test experiments were obtained from the local market. Circular slices (diameter 9 mm, thickness 1 mm) were aseptically cut from the central part of the potato tubers and placed on edge on the bottom of the sample container of calorimeter A, so that each slice was in full contact with the gas phase. The quantity of potato tissue used in an experiment was approximately 1.2–1.3 g fresh weight ( $\approx 0.25$ g dry wt.).

### 2.3. Dynamic corrections

Dynamic correction procedures were applied in order to calculate the true heat flow and $CO_2$ production rate. The thermal inertia of the two calorimeters is corrected for by using a deconvolution procedure based on the Tian equation [29]

$$\phi = \phi_{obs} + \tau_{cal}\, d\phi_{obs}/dt \quad (1)$$

where $\phi$ is the true heat flow rate, $\phi_{obs}$ the observed heat flow rate, $\tau_{cal}$ the calorimetric time constant and $t$ the time. In a similar manner, the true rate of $CO_2$ production in the main calorimeter, in mol $s^{-1}$, can be related to the rate at which $CO_2$ enters the analyzer ($\dot{\xi}_{obs}$), then

$$\dot{\xi} = \dot{\xi}_{obs} + \tau_{CO_2}\, d\dot{\xi}_{obs}/dt \quad (2)$$

Because $\dot{\xi}_{obs}$ can be substituted by $\phi/\Delta H_{CO_2}$, Eqs. (1) and (2) can be combined into an expression for $\dot{\xi}$ as a function of $\phi_{obs}$, the observed heat flow in the $CO_2$ analyzer

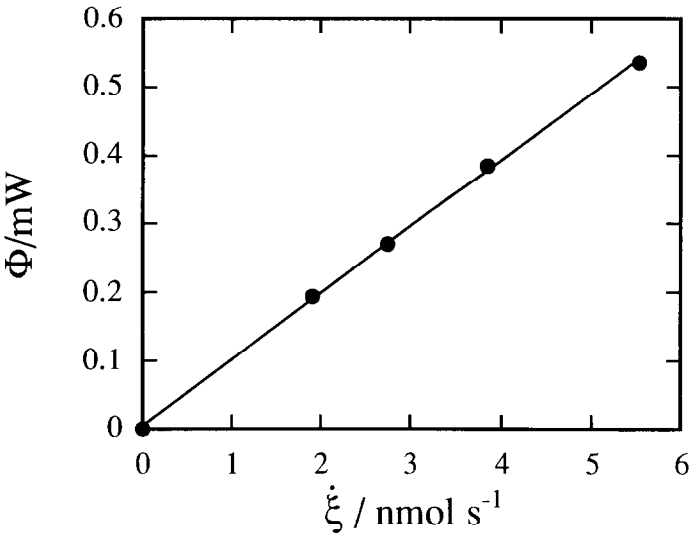


Fig. 3. Calibration curve showing the steady state heat flow rate ($\phi$) as measured by calorimeter B (the $CO_2$ analyzer), as a function of the rate of $CO_2$ injection ($\dot{\xi}$) in the sample chamber of calorimeter A. A linear regression analysis gives a slope corresponding to $a\Delta_r H_{CO_2} = -97$ kJ mol$^{-1}$.

$$\dot{\xi} = 1/\Delta H_{CO_2}[\phi_{obs} + (\tau_{CO_2} + \tau_{cal,B})\, d\phi_{obs}/dt + \tau_{CO_2}\, \tau_{cal}\, d^2\, \phi_{obs}/dt^2] \quad (3)$$

where $\tau_{cal,B}$ is the thermal time constant of calorimeter B, the $CO_2$ analyzer. Although the time constants in Eq. (3) have a physical meaning, and as such can be derived on theoretical grounds, they are in practise derived from calibration experiments.

## 3. Results and discussion

### 3.1. Properties of the main calorimeter

Typically, the long-term baseline stability (24 h) of the main calorimeter (A) was within $\pm 1$ μW. Short-term disturbances normally averaged $\pm 0.1$ μW.

A minor endothermic shift, about 3 μW, was observed at the highest attainable flow rate (150 ml h$^{-1}$) as compared to the baseline position under non-perfusion conditions. The shift decreased with decreasing flow rate. At the rate normally used (45 ml h$^{-1}$), the repeatability of the baseline position between experiments was $\pm 1$ μW. The time constant for calorimeter A ($\tau_{cal,A}$) charged with potato slices was found to be 170 s.

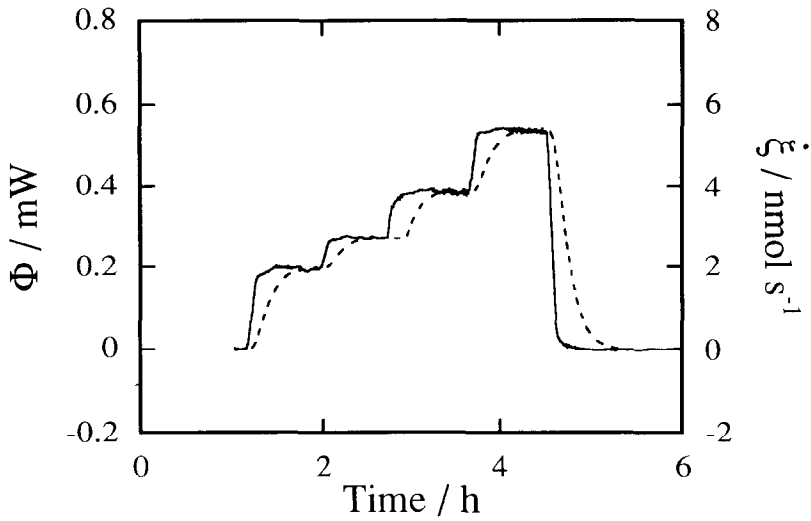


Fig. 4. Output of calorimeter B (the $CO_2$ analyzer) during a calibration experiment. The $CO_2$ injection rate ($\dot{\xi}$) was varied in four steps from zero to 5.5 nmol $s^{-1}$ and then returned to zero. The dashed curve shows the uncorrected results (in mW), whereas the solid curve is corrected for the inertia of the system using Eq. (3).

### 3.2. *On-line $CO_2$ measurements*

Fig. 3 shows results of calibration experiments with the $CO_2$ analyzer (calorimeter B) using $N_2/CO_2$ gas mixtures. It is seen that a linear relationship exists between the $CO_2$ injection rate ($\dot{\xi}$) and the measured steady-state heat flow rate ($\phi$) due to solution/neutralization of $CO_2$. The slope of the line in Fig. 3 corresponds to the molar enthalpy change for the sum of dissolution and neutralization of $CO_2$. The obtained value, $\Delta_r H_{CO_2} = -97$ kJ $mol^{-1}$, is close to that calculated from tabulated data [33] ($-108$ kJ $mol^{-1}$). The long-term baseline stability and short-term irregularities of the signal from calorimeter B were typically $\pm 1$ μW per 24 h and $\pm 0.2$ μW, respectively. From these values, we conclude that the sensitivity of $CO_2$ measurements under steady-state conditions is $\pm 2$ pmol $s^{-1}$. These values can be compared with those obtained with the closed system of Criddle et al. [26,27].

When the concentration of $CO_2$ in the gas flow changes rapidly, the calorimetric signal should be corrected for the total inertia of the calorimetric system, i.e., both for the thermal inertia (of calorimeter B, $\tau_{cal,B}$) and for the inertia with respect to the flow of $CO_2$ from the sample chamber of calorimeter A to the $CO_2$ trap in calorimeter B, $\tau_{CO_2}$ (Eq. (3)). The values for these two time constants were found to be $\tau_{cal,B} = 170$ s and $\tau_{CO_2} = 500$ s. The largest contribution to the overall inertia for the $CO_2$ measurements is thus due to the slow diffusion/washing-out of $CO_2$ in the flow line. Fig. 4, curve a, shows uncorrected results from a calibration experiment where the $CO_2$ injection rate was increased in four steps from zero to

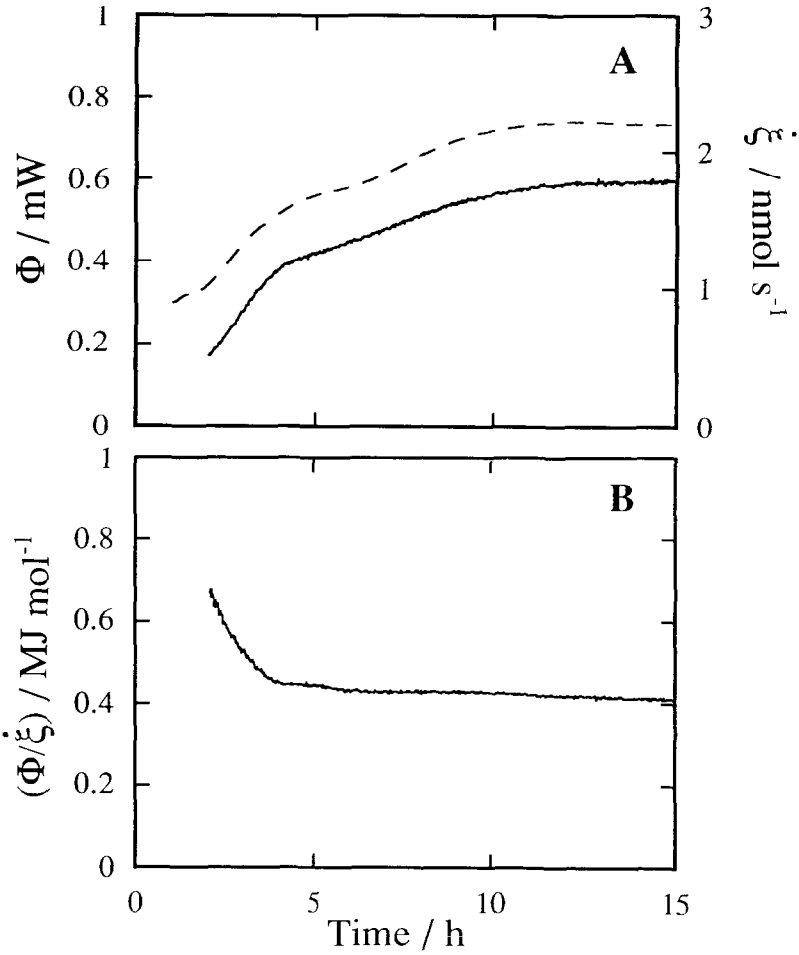


Fig. 5. A. Heat flow rate ($\phi$) and $CO_2$ production rate ($\dot{\xi}$) of 0.25 g (dry wt.) of potato tuber slices. The slices were cut from the potato tuber at time $t = 0$. B. Ratio of heat flow to $CO_2$ production.

5.5 nmol $s^{-1}$ and then returned to zero. Also shown in Fig. 4 is the deconvoluted curve. It is seen that this latter curve shows nearly correct kinetics. As expected, zero and steady-state values, as well as the integrated areas under the two curves, are identical.

### *3.3. Metabolic experiments*

*Potato slices*

As has been observed for many years [30], the respiratory rate of thin potato tuber, depicted in Fig. 5A increases more than three-fold over the time course of the

experiment. This is reflected in both the heat flow rate ($\phi$) and the $CO_2$ production rate ($\dot{\xi}$) of the slices. However additional information can be obtained from calorespirometric data. One of the additional results obtainable is presented in Fig. 5B where a continuous dynamic ratio of the heat flow rate to the $CO_2$ production rate ($\phi\dot{\xi}^{-1}$) is displayed. It can be seen that during the hours shortly after slicing this ratio is high. As the respiratory rate continues to increase the ratio $\phi\dot{\xi}^{-1}$ declines to a nearly constant value of $-450$ kJ mol$^{-1}$, corresponding closely to the value expected for the respiration of carbohydrate. Both the increasing absolute rates and the changing ratio, $\phi\dot{\xi}^{-1}$, indicate that the metabolic processes of the slices are changing as they age. The higher value of $\phi\dot{\xi}^{-1}$ during the early phase indicates that a more reduced substrate is being metabolized or that oxidation of the substrate is incomplete. The observed pattern is consistent with the finding that lipids are respired during the early stage of the aging process [34] followed by a transition to respiration of carbohydrate [35,36]. None of these findings regarding the aging of potato tuber slices are by any means novel, rather they demonstrate the validity of calorespirometry for characterizing the behavior of the potato tuber slice system and thus, its value for studying less-well-known biological phenomena.

Simultaneous measurements of $O_2$ consumption rates would provide additional information on the dynamic $RQ$ ($\dot{\xi}_{CO_2}\dot{\xi}_{O2}^{-1}$) and changes in the energy efficiency of respiration, for example as the alternate oxidase is increasingly engaged during the aging of the slices [30,37]. As yet, a satisfactory $O_2$ measuring system has not been developed for the perfusion calorimeter. Determinations of $O_2$ concentration must be conducted with high precision, because they are quite small. Unlike the case in the closed system [26,27], it is not possible to employ precise pressure measurement techniques to obtain rates of $O_2$ consumption. Furthermore, from an analytical point of view the perfusion techniques have a disadvantage because small changes in the near steady-state concentration of $O_2$ in the effluent stream must be measured. In comparison, in closed systems the concentration changes are accumulative and rates may be determined from changes in the slope of the concentration change. Nonetheless, the perfusion system has important advantages. Unlike the closed system, accumulation of $CO_2$, or other volatile metabolic products that may inhibit respiration or otherwise alter the metabolism or physiology of the tissue [20–23], can be avoided. Furthermore, the composition of the input gas stream may be purposefully altered to introduce gases and study their effects on metabolism. It should be possible, as well, to analyze the isotope ratios of the effluent gases online. Such analysis [34,36,38,39] would provide additional information for dynamically determining which pathways of metabolism and respiration are engaged under particular experimental conditions. The perfusion system could also be adapted for studying photosynthetic metabolism in an illuminated cuvette. For these reasons, we are continuing to evaluate techniques for analysis of $O_2$ and isotopes in the effluent gas stream of the perfusion calorimeter in order to fully exploit its capabilities.

## 4. Conclusions

The perfusion instrument presented here is useful for long-term dark experiments with plant tissues. This configuration is potentially useful for measurement under photosynthetic conditions.

The open system prevents the accumulation of volatile products of metabolism and allows for rapid changes in gas composition or the introduction of metabolic affectors.

Dynamic corrections to account for the effect of slow gas diffusion as well as the thermal inertia of the calorimeter should be applied when rapid changes in the rate and/or quality of metabolism are measured.

The sample chamber normally contains tissue wetted with some medium. In order to minimize uncontrolled evaporation or condensation effects the equilibration cups should be charged with the same medium or at least a medium closely approximating the sample vapor pressure.

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