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ENGINEERING OF GAS PHASE MANAGEMENT AND CONTROL FOR METABOLIC CHAMBERS.

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1 INTRODUCTION

The scope of this Technical Note is to review available mathematical decription, basic laws and equations for modelling and instrumentation for characterising the gas phase balances entering and exiting from a closed chamber where a biological transformation is occurring (metabolic chamber). The chamber can be either a greenhouse (where higher plants are growing) or a room where humans or animals have been installed. Therefore, two kinds of information are reviewed, firstly theoritical and technical information regarding the instrumentation and modelling and, secondly the use of the measurements performed in terms of assessment of the process variables.

This study concerns, from one hand, the derivation of the gas phase model equations, including perfectly mixed assumptions and the influence of the gas phase residence time distribution and, from the other hand, the technical recommendations that can be made for the selection instrumentation both for the control of operating parameters and the assessment of the variables that are useful for the diagnostic of the chamber behavior.

The six first sections of this TN concern theoritical and technical information applicable for both greenhouses design and closed habitats. The two following sections concern the application for closed habitats and greenhouses. Conclusive remarks are developped in the las ninth section.

2 MASS AND ENERGY BALANCES

2.1 General equation

Let us denote by X a certain amount of quantity (mass, energy, momentum...etc.), the general law of conservation, on which all phenomenological descriptions of change are based, reads as follows :

$$\text{(Accumulation of X in system)} = \frac{d}{dt}(X) =$$

$$\sum \text{(Flow of X into system)} - \sum \text{(Flow of X out of system)} + \text{(Production of X in system)} \qquad \text{(Eq. 1)}$$

All terms of previous equation are in unit of quantity of X per unit time, which means that flows are flow rates and that production denotes for a production rate (source term).

The system may be an apparatus, a part of an apparatus, an infinitely element of volume...etc. The production rate may be positive (actual production of X in the system) or negative, *i.e.* consumption of X in the system.

In transient state, the knowledge of the flows of X into and out of system and of the production rates of X enables to determine the evolution of X during time from an initial condition that is known.

At steady state, the quantity of X in the system is constant by definition ; the knowledge of the flows enables to calculate the production rates. If all three terms on the right hand of Eq. 1 are known (or independently measured), the application of the previous balance equation must be done with data reconciliation techniques in order to calculate the best estimation of the measured variables with respect of the steady state assumption.

For further applications it must be kept in mind that Eq. 1 applies whatever the system is perfectly mixed or not.

2.2 Mass balance

The general concept held by previous Eq. 1 is applied to gas balance in a close chamber. The notation are given in Figure 1 and unambiguously listed at the end of this Technical Note. Normally, the matter balance equation must be written in terms of mass or moles and never in terms of volume for a gas phase. Also, it has to be expressed for all components entering, leaving or accumulating in the system. In terms of molar balance, this leads to :

$$\frac{dn_i}{dt} = \boldsymbol{\Phi}_n^{(1)} \boldsymbol{y}_i^{(1)} - \boldsymbol{\Phi}_n^{(2)} \boldsymbol{y}_i^{(2)} + \boldsymbol{\Phi}_L^{(1)} \boldsymbol{c}_i^{(1)} - \boldsymbol{\Phi}_L^{(2)} \boldsymbol{c}_i^{(2)} + \boldsymbol{R}_{ni} \qquad \text{(Eq. 2)}$$

In a reduced form this leads to :

$$\frac{dn_i}{dt} = \boldsymbol{\Delta}\ (\boldsymbol{\Phi}_n\, y_i) + \boldsymbol{\Delta}\ (\boldsymbol{\Phi}_L\, c_i) + \boldsymbol{R}_{ni} \qquad \text{(Eq. 3)}$$

where $\boldsymbol{\Delta}$ operator represent the difference between inlet and outlet variables.

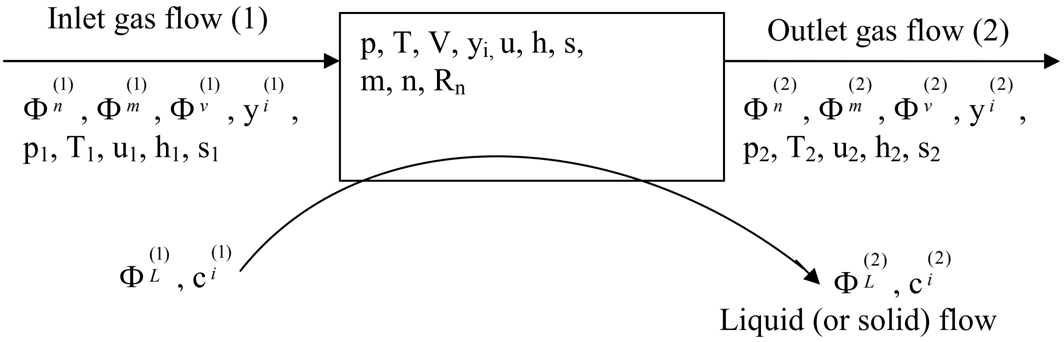


Figure 1 : General scheme and notation for gas matter balance

The mass balance equation is readily obtained by multiplying each term of the previous equation by the molecular weight of component ***i*** (M_i).

Focus on 3 points

- It clearly appears from the above that gas phase balance cannot be considered independently and without considering liquid phase balance.

- Further analysis of matter balance equations for the gas involves an equation of state for the gas phase. It will be considered in all following developments that ideal gas law applies, knowing that more sophisticated equations (Van der Waals, Redlich & Kwong, Peng Robinson) are possible but render the calculation much more complex. For air-based gas compositions, the ideal law approximation does not impair the theoretical calculations (error less than 1 %) for pressures ranging between 0 and 1000 kPa (10 bars) and temperatures ranging between –10 °C and + 50 °C. This assumption also leads to consider that gas enthalpy is only depending on temperature (not on pressure) via proportionality constant which is the heat capacity. For a gas phase which may contain predominantly other gas (such as CO_2), this simplification may lead to much more important errors that have to be considered. All the relations resulting from ideal gas law assumption are presented in Tables 1a to 1d.

- The gas matter balance must be written in terms of moles or mass (and never in terms of volume) for all the components travelling in system. In reactive systems, which is the case for either a greenhouse or a metabolic chamber, the production term R_{ni} (or source term) contains the kinetic model. It must be emphasised that the sum of the source terms for all components on a molar basis is generally not equal to 0 (non conservation of total number of moles) but is equal to 0 when it is calculated on a mass basis (total mass conservation, principle). This last condition (Lavoisier’s mass conservation principle) is even more constrained when applied to each of the elements of the system which absolutely must be done. This is the basic reason why stoichiometric description is systematically used for MELiSSA compartments description. Mathematically this is translated in the set of equations reported in Table 2a and 2b.

<table>
<tr><th colspan="2">Volume, mass, moles quantities</th></tr>
<tr><td>Departure relation</td><td>$pV = n\,R\,T$</td></tr>
<tr><td>Standard conditions</td><td>$T = 0°\text{C} = 273.15\ \text{K}$;
$p = 1\ \text{atm} = 101.3\ \text{kPa}$;
$R = 8.3143\ \text{J.mole}^{-1}.\text{K}^{-1}$;
$v = 2.2414.10^{-2}\ \text{sm}^3.\text{mole}^{-1}$ whatever the gas</td></tr>
<tr><td>Density</td><td>$\rho = \dfrac{p\,M}{R\,T}$ (kg.m^{-3})</td></tr>
<tr><td>Volumic flow</td><td>$\Phi_v = \Phi_n\ \dfrac{R\,T}{p}$ (m^3.s^{-1})</td></tr>
<tr><td>Standard volumic flow</td><td>$\Phi_{vs} = 2.2414.10^{-2}\ \Phi_n$ (sm^3.s^{-1})</td></tr>
<tr><td>Mass flow</td><td>$\Phi_m = M\,\Phi_n$
$\Phi_m = \rho\ \Phi_v = \dfrac{p\,M}{R\,T}\ \Phi_v$</td></tr>
<tr><th colspan="2">Expressions of thermodynamic functions

Let $h_r(T_r)$ and $s_r(T_r,\ p_r)$ the arbitrary values of enthalpy and entropy at an arbitrary reference state at T_r and p_r</th></tr>
<tr><td>Enthalpy (per unit mass)</td><td>$h = h_r + C_p\ (T - T_r)$ (J.kg^{-1})</td></tr>
<tr><td>Internal energy (per unit mass)</td><td>$u = h_r + C_p\ (T - T_r) - \dfrac{R\,T}{M}$ (J.kg^{-1})</td></tr>
<tr><td>Entropy (per unit mass)</td><td>$s = s_r + C_p\ Ln\dfrac{T}{T_r} - \dfrac{R}{M}\ Ln\dfrac{p}{p_r}$ (J.kg^{-1}.K^{-1})</td></tr>
<tr><td>Gibbs energy (per unit mass)</td><td>$\mu = h - T\,s$ (J.kg^{-1})</td></tr>
<tr><th colspan="2">Energy flows</th></tr>
<tr><td>Enthalpy</td><td>$\Phi_h = \Phi_m\ h$</td></tr>
<tr><td>Entropy</td><td>$\Phi_s = \Phi_m\ s$</td></tr>
<tr><td>Gibbs energy</td><td>$\Phi_g = \Phi_m\ \mu$</td></tr>
</table>

Table 1a : Ideal gas law for a pure gas phase

Characterisation of the gas composition	
Departure relation	$p_i \, V = n_i R \, T$
Dalton law	$\sum_i p_i = p$ $n = \sum_i n_i$
Mole fraction	$y_i = \frac{n_i}{n} = \frac{p_i}{p}$
Mass fraction	$\omega_i = \frac{y_i M_i}{\sum_j y_j M_j}$
Molecular weight	$M = \sum_i y_i M_i$ $\omega_i = y_i \frac{M_i}{M}$
Partial molar flow	$\Phi_{ni} = y_i \; \Phi_n$ $\Phi_{ni} = \frac{p_i}{R\,T} \; \Phi_v$
Partial mass flow	$\Phi_{mi} = M_i \; \Phi_{ni}$ $\Phi_{mi} = \omega_i \; \Phi_m$ $\Phi_{mi} = \frac{p_i M_i}{R\,T} \; \Phi_v = \omega_i \frac{p\,M}{R\,T} \; \Phi_v$

Table 1b : Ideal gas mixture : characterisation of the composition

Expressions of thermodynamic functions	
Heat capacity	$C_p = \sum_i \omega_i C_{pi}$ (J.kg^{-1}.K^{-1})
Enthalpy (per unit mass) (all expressions equivalent)	$h = \sum_i \omega_i h_i$ (J.kg^{-1}) $h = \sum_i \omega_i h_{ri} + \sum_i \omega_i C_{pi} (T - T_r)$ (J.kg^{-1}) $h = h_r + C_p (T - T_r)$
Entropy (per unit mass) (all expressions equivalent)	$s_i^0 = s_{ri} + C_{pi} \, Ln \frac{T}{T_r} - \frac{R}{M_i} Ln \frac{p}{p_r}$ (J.kg^{-1}.K^{-1}) $s_i = s_{ri} + C_{pi} \, Ln \frac{T}{T_r} - \frac{R}{M_i} Ln \frac{p_i}{p_r}$ $s_i = s_i^0 - \frac{R}{M_i} Ln \frac{p_i}{p}$ $s = \sum_i \omega_i s_i$ $s = \sum_i \omega_i s_i^0 - \frac{R}{M} \sum_i y_i \, Ln \, y_i$ $s = \sum_i \omega_i (s_i^0 - \frac{R}{M_i} Ln \, y_i)$ $s = \sum_i \omega_i s_{ri} + C_p \, Ln \frac{T}{T_r} - \frac{R}{M} Ln \frac{p}{p_r}$
Gibbs energy (per unit mass)	$\mu = h - T s$ (J.kg^{-1}) $\mu_i^0 = h_i - T s_i^0$ $\mu_i = h_i - T s_i$ $\mu_i = h_i - T \mathrm{s}_i^0 + \frac{RT}{M_i} Ln \, y_i$ $\mu = \sum_i \omega_i (\mu_i^0 - \frac{RT}{M_i} Ln \, y_i)$

Table 1c : Ideal gas mixtures : expressions of the thermodynamic functions

Total energy flows	
Enthalpy	$\Phi_h = \Phi_m\ h$ $\Phi_h = \sum_i\ \Phi_{mi}\ h_i$
Entropy	$s_i^0 = s_{ri} + C_p\ Ln\ \frac{T}{T_r}\ -\ \frac{R}{M_i}\ Ln \frac{p}{p_r}$ $s_i = s_{ri} + C_p\ Ln\ \frac{T}{T_r}\ -\ \frac{R}{M_i}\ Ln \frac{p_i}{p_r}$ $\Phi_s = \Phi_m\ s\ = \sum_i\ \Phi_{mi}\ s_i$ $\Phi_s = \sum_i\ \Phi_{mi}\ (s_i^0 - \frac{R}{M_i}\ Ln\ y_i) = \sum_i\ \Phi_{mi}\ s_i$
Gibbs energy	$\Phi_g = \Phi_m\ \mu = \sum_i\ \Phi_{mi}\ \mu_i$ $\Phi_g = \sum_i\ \Phi_{mi}\ (\mu_i^0 + \frac{RT}{M_i}\ Ln\ y_i)$ $\mu_i^0 = h_i - T\ s_i^0$ $\mu_i\ = h_i - T\ s_i^0 + \frac{RT}{M_i}\ Ln\ y_i$ $\mu_i = M_i\ (h_i - T\ s_i^0) + RT\ Ln\ y_i$

Table 1d : Ideal gas mixtures : total energy flows

Notation		
$\mathbf{R_n}$ (n, 1)	Column vector of the n transformation rates of the n compounds	n values R_{ni} (mole.s^{-1})
R (r, 1)	Column vector of the r rates associated to the r stoichiometries used to describe the transformations	r values R_r (mole.s^{-1})
$\boldsymbol{\alpha}$ (n, r)	(n, r) matrix of the stoichiometric coefficients : reactions in columns	e . r values α_{ik}
$\boldsymbol{\beta}$ (e, n)	(e, n) matrix of the elements composition of the n compounds. Elements in line. Compounds in column	e . n values β_{ji}
$\mathbf{M_n}$ (n, 1)	Column vector of the molecular weight of the n compounds.	n values M_i (g.mole^{-1} or kg.mole^{-1})
$\mathbf{M_e}$ (n, 1)	Column vector of the molecular mass of the elements.	e values M_j (g.mole^{-1} or kg.mole^{-1})

Table 2a : General matricial representation of mass conservation principles : definition of notation

<table>
<tr><th colspan="2">Calculation of compounds transformation rates</th></tr>
<tr><td>$\mathbf{R_n} = \boldsymbol{\alpha} \,.\, \mathbf{R}$</td><td>$R_{ni} = \sum_k \alpha_{ik} R_k$ (i = 1, n)</td></tr>
<tr><th colspan="2">Elements conservation principle (Lavoisier's principle)</th></tr>
<tr><td>$\boldsymbol{\beta} \,.\, \mathbf{R_n} = 0$</td><td>$\sum_i \beta_{ji} R_{ni} = 0$ (j = 1, e)</td></tr>
<tr><th colspan="2">Contraints on kinetic rates values</th></tr>
<tr><td>$\boldsymbol{\beta} \,.\, \boldsymbol{\alpha} \,.\, \mathbf{R} = 0$</td><td>$\sum_i \sum_k \beta_{ji} \alpha_{ik} R_k = 0$ (j = 1, e)</td></tr>
<tr><th colspan="2">Molecular weight calculation</th></tr>
<tr><td>$\mathbf{M_n} = \boldsymbol{\beta}^T \,.\, \mathbf{M_e}$</td><td>$M_i = \sum_j \beta_{ji} M_j$ (i = 1, n)</td></tr>
<tr><th colspan="2">Total mass conservation (weaker contrainst)</th></tr>
<tr><td colspan="2">$\mathbf{M}_e^T = \boldsymbol{\beta} \,.\, \boldsymbol{\alpha} \,.\, \mathbf{R} = 0$</td></tr>
<tr><td colspan="2">$\mathbf{M}_n^T = \mathbf{M}_e^T \,.\, \boldsymbol{\beta}$</td></tr>
<tr><td colspan="2">$\mathbf{M}_n^T \,.\, \boldsymbol{\alpha} \,.\, \mathbf{R} = \mathbf{M}_n^T \,.\, \mathbf{R_n} = 0$
$\sum_i M_i R_{ni} = 0$</td></tr>
</table>

Table 2b : General matricial presentation of matter conservation principle : conservation relations

2.3 *Energy balances*

According to the second principle of thermodynamics, the energy balances must set two independent equations : the first one is the total energy balance that is the first principle of thermodynamics ; the second one is obtained considering a special comptability of mechanical energy that is issued from the second principle of thermodynamics.

2.3.1 TOTAL ENERGY BALANCE

The first principle of thermodynamics or total energy balance is written as follows :

$$\frac{d}{dt}(U + E_{cin} + E_{pot}) = \Delta\ [\Phi_m\ (\frac{1}{2} v^2 + g\,z + h\ )] + \Phi_{mech} + \Phi_{heat} + \Phi_{rad} \qquad \text{(Eq. 4)}$$

where the operator Δ denotes for (inlet – outlet). The Δ terms is often called the convective term. Φ_{mech} represents the mechanical power input in the system (Φ_{mech} is in watts and positive if it is an input in the system and negative if it is produced by the system). Φ_{heat} is the thermal power input in the system (Φ_{heat} is in watts and positive if it is an input in the system and negative if it is heat produced by system and released by other way than convective term). Φ_{rad} is the radiative power input in the system and is expressed in watts. Generally, the previous Eq. 4 can be simplified, considering that the accumulation of kinetic and potential energy in the system (E_{cin} and E_{pot}) are negligible, so that :

$$\frac{dU}{dt} = \Delta\ [\Phi_m\ (\frac{1}{2} v^2 + g\,z + h\ )] + \Phi_{mech} + \Phi_{heat} + \Phi_{rad} \qquad \text{(Eq. 5)}$$

where v and z denotes for the gas velocity and the height at inlet and outlet of the system and g for terrestrial gravity.

2.3.2 MECHANICAL ENERGY BALANCE

In the general case of all variables varying in space and time, the mechanical energy balance cannot be written simply in an integral form. However in relevant practical cases, simple expressions can be derived.

<u>Steady state for all variables describing the system.</u>

$$\Phi_m\ \Delta\ [\frac{1}{2} v^2 + g\,z\ ] - \Phi_m \int_{input}^{output} \Phi_m \frac{dp}{\rho}\ +\ \Phi_{mech} - \Phi_{frict} = 0 \qquad \text{(Eq. 6)}$$

where Φ_{frict} denotes for the mechanical power dissipated by friction losses.

For isothermal operation (same temperature at input and output) and ideal gas assumption, the previous Eq. 6 becomes :

$$\Phi_m \, \Delta \left[\frac{1}{2} v^2 + g\,z\right] + \Phi_m \frac{RT}{M} \, Ln \, \frac{p_{input}}{p_{output}} + \Phi_{mech} - \Phi_{frict} = 0 \qquad \text{(Eq. 7)}$$

That is the Bernouilli equation.

2.3.3 USEFUL EQUATIONS

From the above it clearly appears that the energy conservation principles are held by two independent equations : total energy balance and mechanical energy balance. Also these equation involve non convective energy flows terms Φ_{mech}, Φ_{heat}, Φ_{frict} and Φ_{rad} that must be calculated or modelled using physical transfer phenomenological description. This involves momentum transfer for modelling Φ_{mech} and Φ_{frict}, heat transfer for Φ_{heat} and radiative transfer for Φ_{rad}. In practice, simplifying asssumptions may be used for some of these basic physical phenomena generally letting only one rate limiting process to be described in detail.

Moreover, in most case, it is possible to oversimplify the previous balance equations considering only the dominant phenomena.

Invariable pressure (constant pressure in time) but eventually not in space.

$$\frac{dH}{dt} = \Delta\,[\Phi_m \, h] + \Phi_m \int_{input}^{output} \Phi_m \frac{dp}{\rho} + \Phi_{heat} + \Phi_{rad} + \Phi_{frict} \qquad \text{(Eq. 8)}$$

That is the thermal balance (enthalpic balance) which is only valid for constant pressure in time. That is obtained by difference between total energy balance and mechanical enregy balance.

Constant temperature in space and time : isothermal systems.

When the temperature in system remains constant, the expression of the difference between total energy balance and mechanical energy balance leads to a simplified expression of the accumulation term which represents the accumulation of entropy in system. In that case, the so called entropy balance can be written as follows.

$$T \frac{dS}{dt} = \Delta\,[\Phi_m \, s] + \Phi_{heat} + \Phi_{rad} + \Phi_{frict} \qquad \text{(Eq. 9)}$$

That is the entropy balance which directly refers to accumulation of entropy inside the system.

<u>Isentropic systems</u>.

If there is no change of entropy between input and output flows, the system is isentropic. The necessary and sufficient condition for the system to be isentropic is obtained from the difference between total energy and mechanical energy balances, whatever the system is at steady state or transient state. It is written as follows :

$$\Phi_{heat} + \Phi_{rad} + \Phi_{frict} = 0 \qquad \text{(Eq. 10)}$$

The previous equation enables to define a necessary and sufficient condition for having an isentropic system which means that the entropy of the system remains constant in time. That is by example the departure assumption for all gas compressors.

Application to metabolic chambers

Generally, for the gas phase of a metabolic chamber, it can be supposed that the hydrodynamics is at steady state (no transient pneumatic representation of the gas flows) so that Bernoulli equation (Eqs. 6 or 7) applies. It must be kept in mind that starting and stop procedures of pumps and valves cannot be represented by such an equation.

At steady state or more exactly at pseudo-steady state, the friction losses Φ_{frict} can therefore be calculated and the result is introduced in either enthalpic or entropic balances. The isothermal assumption for calculating the pressure term cannot be done in general. However a good numerical approximation is obtained by using an average temperature between input and output which renders the calculation possible.

2.4 Assessment of gas production and consumption rates

The previous developments relate the general form of the basic equations relevant for matter and energy balances.

The departure relation is the molar balance Eq. 2 applied independently for all components of the gas phase. In order to use it in practice, we propose to establish a classification of the assumptions relative to flow regimes, homogeneity, gas exchanges between phases...etc. which enables to gradually improve the accuracy of the model which has to developped from the simplest views (but sometimes of poor accuracy) to more evolved ones which would be more relevant of actual behaviors of metabolic chambers.

Several classes of models are proposed in Table 3.

Model	**Component**	**Regime**	**Gas flow**	**Liquid flow**	**Gas/liquid equilibration**	**Liquid phase**	**Gas phase homogeneity**	**Reference Equations**
0 order model	N_2 (or inert gas), O_2, CO_2	Steady state or pseudo steady state	Yes	No	No transfer	No	T, p, y_i homogeneous	Eqs. 11 to 17
1st order model	N_2 (or inert gas), O_2, CO_2, H_2O	Steady state or pseudo steady state	Yes	No	Only water evaporation or condensation	No	T, p, y_i homogeneous	Eqs. 18 to 23
2nd order model	N_2 (or inert gas), O_2, CO_2, H_2O	Steady state or pseudo steady state	Yes	Yes water flow and production into chamber	Only water evaporation or condensation	Yes water balance	T, p, y_i homogeneous	Eq. 24
3rd order model	N_2 (or inert gas), O_2, CO_2, H_2O	Transient state	Yes	Yes water flow only	Gas/liquid water equilibrium (pure water)	Yes water balance	T, p, y_i homogeneous	Eqs. 25 and 26
4th order model	All compounds	Steady state or pseudo steady state	Yes	Liquid flow	Gas/liquid equilibria for all components	Yes at equilibrium for all components	T, p, y_i homogeneous	Eqs. 27 and 28
5th order model	All compounds	Transient state	Yes	Liquid flow	Gas/liquid transfer rate	Yes	T, p, y_i homogeneous	Eqs. 29 to 32
6th order model	All compounds	Steady state	Yes	Liquid flow	Gas/liquid transfer rate	Yes	T, p, y_i non homogeneous : several zones defined for gas phase	
7th order model	All compounds	Transient state	Yes	Liquid flow	Gas/liquid transfer rate	Yes	T, p, y_i non homogeneous : several zones defined for gas phase	

Table 3 : Different classes of models for the gas phase of a metabolic chamber.

Zero order model

The respiration rates are defined and calculated from input/output data.

$$\phi_n = \frac{p}{RT}\ \phi_v = \frac{1}{M}\ \phi_m \qquad \text{(Eq. 11)}$$

$$\boldsymbol{R_{n\,O2}} = \phi_n^{(2)}\ \boldsymbol{y}_{O2}^{(2)} - \phi_n^{(1)}\ \boldsymbol{y}_{O2}^{(1)} \qquad \text{(Eq. 12)}$$

$$\boldsymbol{R_{n\,CO2}} = \phi_n^{(2)}\ \boldsymbol{y}_{CO2}^{(2)} - \phi_n^{(1)}\ \boldsymbol{y}_{CO2}^{(1)} \qquad \text{(Eq. 13)}$$

$$\boldsymbol{R_{n\,N2}} = \phi_n^{(2)}\ \boldsymbol{y}_{N2}^{(2)} - \phi_n^{(1)}\ \boldsymbol{y}_{N2}^{(1)} \qquad \text{(Eq. 14)}$$

Considering the sum equations :

$$\boldsymbol{y}_{N2}^{(1)} + \boldsymbol{y}_{O2}^{(1)} + \boldsymbol{y}_{CO2}^{(1)} = \boldsymbol{1} \qquad \text{(Eq. 15a)}$$

$$\boldsymbol{y}_{N2}^{(2)} + \boldsymbol{y}_{O2}^{(2)} + \boldsymbol{y}_{CO2}^{(2)} = \boldsymbol{1} \qquad \text{(Eq. 15b)}$$

We obtain :

$$\boldsymbol{R_{O2}} = \phi_n^{(1)} \left[\frac{1 - y_{O2}^{(1)} - y_{CO2}^{(1)}}{1 - y_{O2}^{(2)} - y_{CO2}^{(2)}}\ \boldsymbol{y}_{O2}^{(2)} - \boldsymbol{y}_{O2}^{(1)}\right] \qquad \text{(Eq. 16)}$$

$$\boldsymbol{R_{CO2}} = \phi_n^{(1)} \left[\frac{1 - y_{O2}^{(1)} - y_{CO2}^{(1)}}{1 - y_{O2}^{(2)} - y_{CO2}^{(2)}}\ \boldsymbol{y}_{CO2}^{(2)} - \boldsymbol{y}_{CO2}^{(1)}\right] \qquad \text{(Eq. 17)}$$

The last two equations show that with the measurement of gas flow rate at the intrance of the metabolic chamber $\phi_n^{(1)}$ (or $\phi_m^{(1)}$) and the measurement of O_2 and CO_2 mole fractions at inlet and outlet of the chamber $\boldsymbol{y}_{O2}^{(1)}$, $\boldsymbol{y}_{CO2}^{(1)}$, $\boldsymbol{y}_{O2}^{(2)}$ and $\boldsymbol{y}_{CO2}^{(2)}$, the rates of O_2 and CO_2 production/consumption rates can be calculated

Though very they are very simple, Eqs 16 and 17 are only valid in the framework of the assumptions reported in Table 3 for the zero order model.

First order model

Compared to the previous one, the first-order model brings the water vapour balance on the gas phase. In order to be held properly, the water partial pressure $\boldsymbol{p_w}$ and the relative humidity $\boldsymbol{H_R}$ must be defined.

$$p_w = H_R \; P_w^0(T) \qquad \text{(Eq. 18)}$$

Of course the relative humidity H_R is always less than unity so that : $p_w \leq P_w^0(T)$.

$P_w^0(T)$ is the saturated vapour pressure. It only depends on temperature and not on total gas pressure in the framework of ideal gas approximation. It is accurately represented by Antoine law in the temperature range 0°C - 140°C :

$$Ln\, P_w^0 = A - \frac{B}{T+C} \qquad \text{(Eq. 19)}$$

A = 18.3036
B = 3816.44
C = - 46.13

P_w^0 is expressed in mm Hg in Eq. 19.

The water vapour gas mole fraction is then calculated :

$$y_w = \frac{p_w}{p} = H_R \frac{\mathrm{P}_\mathrm{w}^0}{\mathrm{p}} \qquad \text{(Eq. 20)}$$

The previous equation shows that measurement of relative humidity H_R only makes sense if total (hydrostatic) pressure p and temperature are known at a given point. This is the only way to convert relative humidity into water mole fraction (Eq. 20).

From the above, the sum equations Eqs. 15a and 15b are rewritten accounting for water content :

$$y_{N2}^{(1)} + y_{O2}^{(1)} + y_{CO2}^{(1)} + y_w^{(1)} = 1 \qquad \text{(Eq. 21a)}$$

$$y_{N2}^{(2)} + y_{O2}^{(2)} + y_{CO2}^{(2)} + y_w^{(2)} = 1 \qquad \text{(Eq. 21b)}$$

So that :

$$R_{O2} = \phi_n^{(1)} \left[\frac{1 - y_{O2}^{(1)} - y_{CO2}^{(1)} - \mathrm{y}_\mathrm{w}^{(1)}}{1 - y_{O2}^{(2)} - y_{CO2}^{(2)} - y_w^{(2)}} \, y_{O2}^{(2)} - y_{O2}^{(1)} \right] \qquad \text{(Eq. 22)}$$

$$R_{CO2} = \phi_n^{(1)} \left[\frac{1 - y_{O2}^{(1)} - y_{CO2}^{(1)} - y_w^{(1)}}{1 - y_{O2}^{(2)} - y_{CO2}^{(2)} - y_w^{(2)}} \, y_{CO2}^{(2)} - y_{CO2}^{(1)} \right] \qquad \text{(Eq. 23)}$$

The previous equations show that $\boldsymbol{R_{O2}}$ and $\boldsymbol{R_{CO2}}$ are more finely approached if $\boldsymbol{H_R}$, $\boldsymbol{p}$ and $\boldsymbol{T}$ are measured at inlet and outlet gas streams in order to assess water vapour content.

In order to be free of this kind of measurements, it is often suitable to use drying gas devices before measuring $\boldsymbol{y}_{O2}$ and $\boldsymbol{y}_{CO2}$ at inlet and outlet streams. Of course, this prevents from measuring $\boldsymbol{T}$, $\boldsymbol{p}$ and $\boldsymbol{H_R}$ but the water trap must be regularly refreshed. In case of drying of gas streams $\boldsymbol{y}_w^{(1)}$ and $\boldsymbol{y}_w^{(2)}$ can be neglected so that Eqs. 16 and 17 remain valid.

Second order model

The previous two models do not account for water balance at the metabolic chamber level. This second-order model further complexifies the previous description with the water balance accounting for a net liquid flow, considered as pure water.

The respiration rates are always given by Eqs. 22 and 23 or Eqs. 16 and 17 if the gas stream is dried at $\boldsymbol{y}_{O2}$ and $\boldsymbol{y}_{CO2}$ measurements points. Water balance is given by :

$$\boldsymbol{R_{H2O}} = \phi_n^{(1)} \left[\frac{1 - y_{O2}^{(1)} - y_{CO2}^{(1)} - \mathrm{y}_{\mathrm{w}}^{(1)}}{1 - y_{O2}^{(2)} - y_{CO2}^{(2)} - y_w^{(2)}} \boldsymbol{y}_w^{(2)} - \boldsymbol{y}_w^{(1)} \right] + \phi_L^{(2)} - \phi_L^{(1)} \qquad \text{(Eq. 24)}$$

Eq. 24 shows that the water balance can be assessed if liquid flows $\phi_L^{(2)}$, $\phi_L^{(1)}$ (here considered as pure water) are measured. In practical cases, there are no special difficulties for doing it except that the metabolic chamber must be strictly closed avoiding any leakage either on liquid or gas flows. This may become a technical problem when the size of the chamber is increased.

Third order model

Coming back to the general mass balance Eq. 2, this third order model introduces accumulation terms. It must be outlined that the nature of the balance equation is changed when compared to the previous models. The mass balance principle leads in this case to a differential equation which must be integrated versus time when all the terms of the right hand side of Eq. 2 are known and when an initial condition is assumed.

In practice, there are as many ordinary differential equations (ODE) as the number of constituents for which the mass balance is written. The result of integration is the modelled time course of system variables (flows, mole fractions, concentrations). These modelled variables are numerically compared to the measured ones. If necessary, some coefficients of the model which are contained in the expression of the right hand side terms of Eq. 2, are identified in order to fit the experimental observations to the model. These unknown coefficients are generally included in the kinetic terms $\boldsymbol{R_{ni}}$ in such a way that identification procedure leads to determine the numerical model of kinetic term. It must be stressed that the data reconciliation procedure is performed with elements conservation constraints when the kinetic rates models use the elements conservation principles as given in Tables 2a, 2b. Obviously, this is mandatory and strongly constraints the identification procedure.

Additionally to the previous relations obtained for other models, the accumulation terms must be expressed.

Considering that the gas phase in the chamber is characterised by the volume $\boldsymbol{V_G}$, the pressure $\boldsymbol{p}$ and the temperature $\boldsymbol{T}$ and the liquid phase (pure water) volume is $\boldsymbol{V_L}$, the accumulation terms (in moles) are as follows :

O_2, CO_2, N_2 balance

$$\textbf{Accumulation} = \frac{d}{dt}\left[y_i \frac{pV_G}{RT}\right] \qquad \text{(Eq. 25a)}$$

H_2O balance

$$\textbf{Accumulation} = \frac{d}{dt}\left[y_w \frac{pV_G}{RT} + \frac{\rho_{H2O}^{(L)} V_L}{M_{H2O}}\right] \qquad \text{(Eq. 26a)}$$

If the volumes and total pressure can be considered as constant in time, the integration leads to obtain the mole fraction versus time. The accumulation terms become :

O_2, CO_2, N_2 balance

$$\textbf{Accumulation} = \frac{pV_G}{RT}\frac{dy_i}{dt} \qquad \text{(Eq. 25b)}$$

H_2O balance

$$\textbf{Accumulation} = \frac{pV_G}{RT}\frac{dy_i}{dt} + \frac{\rho_{H2O}^{(L)}}{M_{H2O}}\frac{dV_L}{dt} \qquad \text{(Eq. 26b)}$$

Also, there exist situations where some constituents can be considered at steady state and other not. This is referred as pseudo-steady state assumptions for the constituents that are not accumulated. The model therefore contains both lower order equations (Eqs. 16 to 24) and accumulation terms (Eqs. 25 and 26) for the constituents that are in transient state. In this case, the main difficulty is to suitably classify the variables choosing the permanent variables and the transient ones.

Fourth order model

This class of model (back to steady state models) introduces partition coefficients for all constituents between gas and liquid phases. This entails that permanent gas compounds (O_2, CO_2, N_2, C_2H_4 …) are actually partially solubilised in liquid phase and that dissolved compounds (such as volatile fatty acids : VFA) may be present in gas phase.

Compared to second-order model, there is a further degree of complexity due to the fact that the composition of the gas is defined for a larger number of compounds. In order to manage with this approach, the G/L partition coefficients must be defined as follows :

$$\boldsymbol{K_i} = \frac{p_i}{c_i} = \frac{y_i}{c_i}\,\boldsymbol{p} \qquad \text{(unit Pa.m}^3\text{.mole}^{-1}\text{)} \qquad \text{(Eq. 27)}$$

Normally $\boldsymbol{K_i}$ is independent of pressure. $\boldsymbol{K_i}$ generally depends on temperature. The determination of $\boldsymbol{K_i}$ must account for liquid phase non ideality (which is the general rule for aqueous solutions) and liquid phase chemical or pseudo-chemical equilibria. By example, in the important case of CO_2, the partition coefficient must account for bicarbonate and carbonate equilibria which in terms depend on pH. The mass balance equation is generalised from Eq. 24 as follows :

$$\boldsymbol{R_{ni}} = \phi_n^{(1)} \left[\frac{y_{inert}^{(1)}}{y_{inert}^{(2)}}\, \boldsymbol{y}_i^{(2)} - \boldsymbol{y}_i^{(1)} \right] + \phi_L^{(2)}\, \boldsymbol{c}_i^{(2)} - \phi_L^{(1)}\, \boldsymbol{c}_i^{(1)} \qquad \text{(Eq. 28)}$$

$\boldsymbol{y_{inert}}$ represents the mole fraction of an inert gas compound which is supposed to be not converted in the chamber and not dissolved in liquid phase. By example inert gas can be Argon or Nitrogen.

Fifth order model

This model presents two further improvements compared to the previous ones :

- physical transfer rate description between gas and liquid phases : this leads to consider that gas and liquid phases are no longer at equilibrium.

- transient state for all or some of the compounds. The G/L transfer rate equation is given using a classical volumetric gas transfer coefficient $\boldsymbol{K_{Li}\ a}$, $\boldsymbol{K_{Li}}$ being the transfer coefficient (depending on the compound normally via the diffusion coefficient) and $\boldsymbol{a}$ being the interfacial area per unit of liquid volume.

$$\boldsymbol{R_{G/Li} = K_{Li}\ a\ (c_i - c_i^*)\ V_L} \qquad \text{(unit mole.s}^{-1}\text{)} \qquad \text{(Eq. 29a)}$$

$\boldsymbol{c_i^*}$ is the concentration at equilibrium and is given as previously by Eq.27.

$$c_i^* = \frac{y_i p}{K_i} \qquad \text{(Eq. 30)}$$

Also, Eq. 29 is written using the sign convention that $R_{G/Li} > 0$ if compound i enters the gas phase, namely if the transfer is from liquid to gas phase. This is the case when $c_i > c_i^*$. Conversely, if $c_i^* > c_i$, the transfer of compound i occurs from gas phase to liquid phase and $R_{G/Li} < 0$.

It must be outlined that Eq. 29 enables to completely separate gas balance and liquid (or solid) balances so that the general mass balance Eq. 2 is splitted into two independent equations. Assuming that no conversion occurs in gas phase and that all reactions are in the liquid (or solid) phases, Eq. 2 becomes :

Gas phase

$$\frac{d}{dt}\left(y_i \frac{pV_G}{RT}\right) = \phi_n^{(1)}\, y_i^{(1)} - \phi_n^{(2)}\, y_i^{(2)} + K_{Li}\, a\, (c_i - c_i^*)\, V_L \qquad \text{(Eq. 31)}$$

Liquid phase

$$\frac{d}{dt}(V_L\, c_i) = \phi_L^{(1)}\, c_i^{(1)} - \phi_L^{(2)}\, c_i^{(2)} + K_{Li}\, a\, (c_i^* - c_i)\, V_L + R_{ni} \qquad \text{(Eq. 32)}$$

It must be emphasized that Eq. 32 may be in fact further splitted into several equations for the same component i in the case several liquid or solid phase are present in the metabolic chamber. This is the case when higher plants are growing leading to separate actual liquid phase which circulates around the roots and the vegetal biomass (leaves, stems, roots….) which accumulates into chamber.

It must also be emphasized that the sum of Eq. 31 and 32 leads back to Eq. 2. This is normal. The main interest of splitting Eq. 2 into Eqs. 31 and 32 is that it is the only way to include in the kinetic terms a physical mass transfer rate limiting process. This is an important point because a kinetic limitation (or control) by mass transfer can only be described in the model by a volumetric mass transfer coefficient K_{Li} and a specific exchange area per unit liquid volume a. This is (probably) the case (at least) for water exchanges during the growth of higher plants. This must be taken into account properly for having a chance to develop a knowledge model of the effect of water exchange limitations on higher plant growth.

In principle, there may also exist some cases where gas exchanges with aerial part of plants are rate limiting factors. In order to account for that in a correct way, it is necessary to introduce a gas boundary layer with a mass transfer resistance characterized by a volumetric mass transfer coefficient K_{Gi} and a specific gas exchange area a_G. In that case, instead of Eq. 29a, the exchange rate is expressed as follows :

$$R_{G/Si} = K_{Gi}\, a_G\, (p_i - p_i^*)\, \frac{V_G}{RT} \qquad \text{(unit mole.s}^{-1}\text{)} \qquad \text{(Eq. 29b)}$$

p_i^* is the gas partial pressure of compound i at gas/solid interface. p_i is the bulk partial pressure. K_{Gi} generally depends on total hydrostatic pressure p so that the exchange rate depends on total pressure. This kind of analysis may give a clue for analysing the effect of total pressure on plant growth. This is of interest for considering by example the growth of higher plants in hypobaric chambers.

Sixth order and higher order models

Compared to the previous model, the gas phase in the chamber is no longer considered as homogeneous. The general mass balances are described for each zone with the same type of equations as previously. The main problem consists in the definition of relevant zones for describing the gas phase knowing that the greatest the number of zones, the highest the complexity of the mathematical system and the number of independent variables.

The clue for fixing the number of homogeneous zones is certainly today to use Computer Fluid Dynamics (CFD).

A major contribution is also to finely describe solid/gas/liquid interfaces in order to correctly account for physical rate transfer limited processes. In other words, S/G or S/L interfaces constitute independent interfacial zones which obey special mass and heat transfer description enabling to calculate interface variables such as temperature, partial pressures (and therefore humidity), hydrostatic pressure...etc.

3 TECHNOLOGY DEVELOPMENTS

3.1 Control of system pressure : outlet gas circuit

The basic way to control the system pressure is to act on the outlet gas circuit, as generally done to control the pressure in bioreactors. The basic scheme is shown in Figure 2.

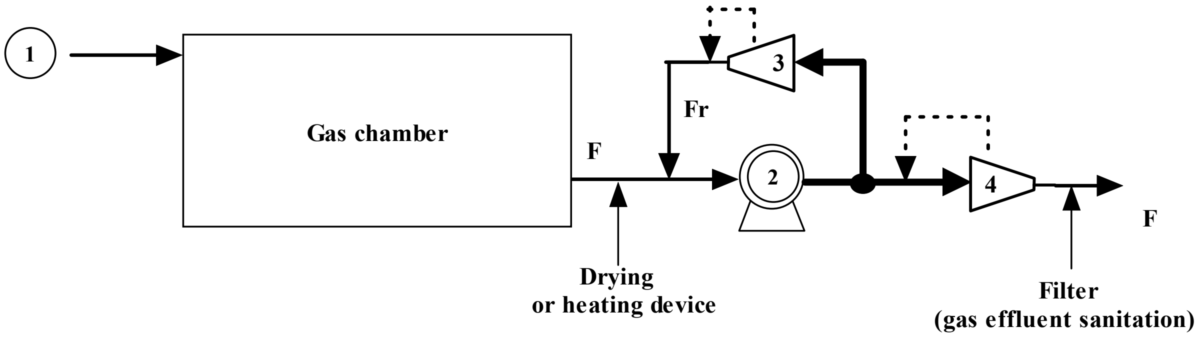


Figure 2 : General scheme of outlet gas circuit

① : Inlet gas circuit
② : Compressor
③ : Downpressure regulator
④ : Back pressure regulator

Bold part of the circuit is the high pressure part ; it is controlled by ④

Assuming that the ratio of recirculated flow F_r is at least 3 times the net gas flow F, ($F_r / F = 3$), the pressure at the intrance of the compressor is controlled by the downpressure regulator by ③. Therefore the pressure in the chamber is controlled provided the pressure drop between chamber and compressor is not too high. Consequently, if gas outlet needs by example to be filtered, it must be done after the backpressure regulator by ④.

It must also be kept in mind that compressing a non-water saturated gas may even lead to water condensation. This may be avoided if the gas is dried before entering the gas outlet circuit or just by limiting the pressure and increasing the temperature in the high pressure part of the circuit.

Water condensation can be easily predicted by calculating the temperature at the output of the compressor 2 (isentropic compression) which enables to calculate the saturated vapor pressure P_w^0 which must be compared to water partial pressure after compression. This supposes to know the water partial pressure before the compressor and therefore the relative humidity into chamber.

From the above, it is clearly shown that the choice of compressor ② and pressure regulators ③ and ④ must be done considering at engineering level the question of flow rate, temperature, condensation and control of humidity.

3.2 Control of flow rate : inlet gas circuit

In order to have a proper assessment of chamber variables, it is important to measure and control the gas flow rate into chamber. The most accurate way is to use mass flow controller. However it must be stressed that this kind of device generally works using gas properties (generally heat capacity) as internal variables in such a way that the output value of the apparatus is based on nitrogen (or air). In case the gas composition deviates significantly from air composition, corrections must be added. Whatever the gas composition, the mass flow controllers have the main advantage to display a gas flow value independent of pressure and temperature.

The general scheme of the inlet gas circuit is given in Figure 3.

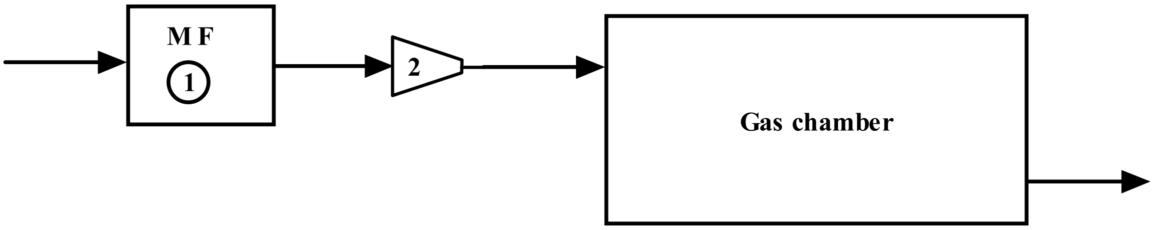


Figure 3 : General scheme of inlet gas circuit.

① : Mass flow controller
② : Down pressure regulator (optional)

The down pressure regulator is optional in normal use if chamber pressure is finely controlled by outlet gas circuit. However, it is useful for starting procedure or in pneumatic transient state.

Whatever the final flowchart chosen, the set of tubes and regulators composed of schemes reported in Figures 2 and 3 must be validated by a simulation of pneumatic behavior of the entire system, especially in transient regime in order to establish a control strategy for pressure, temperature and humidity.

3.3 Control of humidity

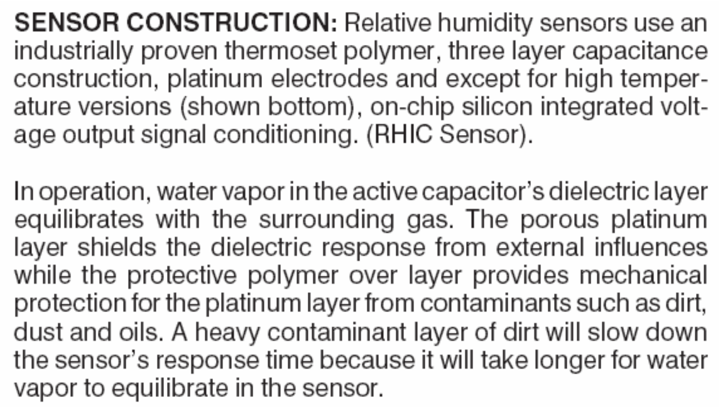
The control of gas humidity can be performed only at a fixed pressure and temperature (see Eqs. 18 - 20), knowing that any variation (in space or in time) changes the relative humidity. There are two parts to examine :

- On-line measurement of H_R
- Regulation and control of humidity

On-line measurement of H_R

Humidity sensors are currently available. It must be outlined that humidity measurement would normally be performed with temperature and pressure to keep a significance of the measurement of H_R.

Choice of a humidity sensor will depend on its accuracy, range of functioning and stability. As temperature affects humidity measure, it would be better to use humidity sensor with integrated temperature sensor for compensation. Honeywell ® would be a possible supplier. The principle and the characteristics of this kind of sensor are described in Figure 4 and Table 4.



SENSOR CONSTRUCTION: Relative humidity sensors use an industrially proven thermoset polymer, three layer capacitance construction, platinum electrodes and except for high temperature versions (shown bottom), on-chip silicon integrated voltage output signal conditioning. (RHIC Sensor).

In operation, water vapor in the active capacitor's dielectric layer equilibrates with the surrounding gas. The porous platinum layer shields the dielectric response from external influences while the protective polymer over layer provides mechanical protection for the platinum layer from contaminants such as dirt, dust and oils. A heavy contaminant layer of dirt will slow down the sensor's response time because it will take longer for water vapor to equilibrate in the sensor.

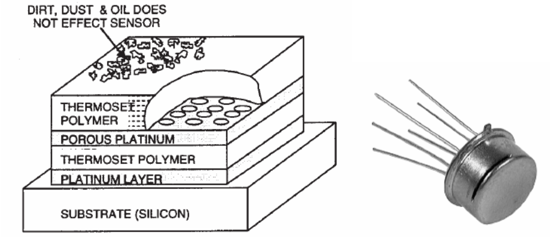
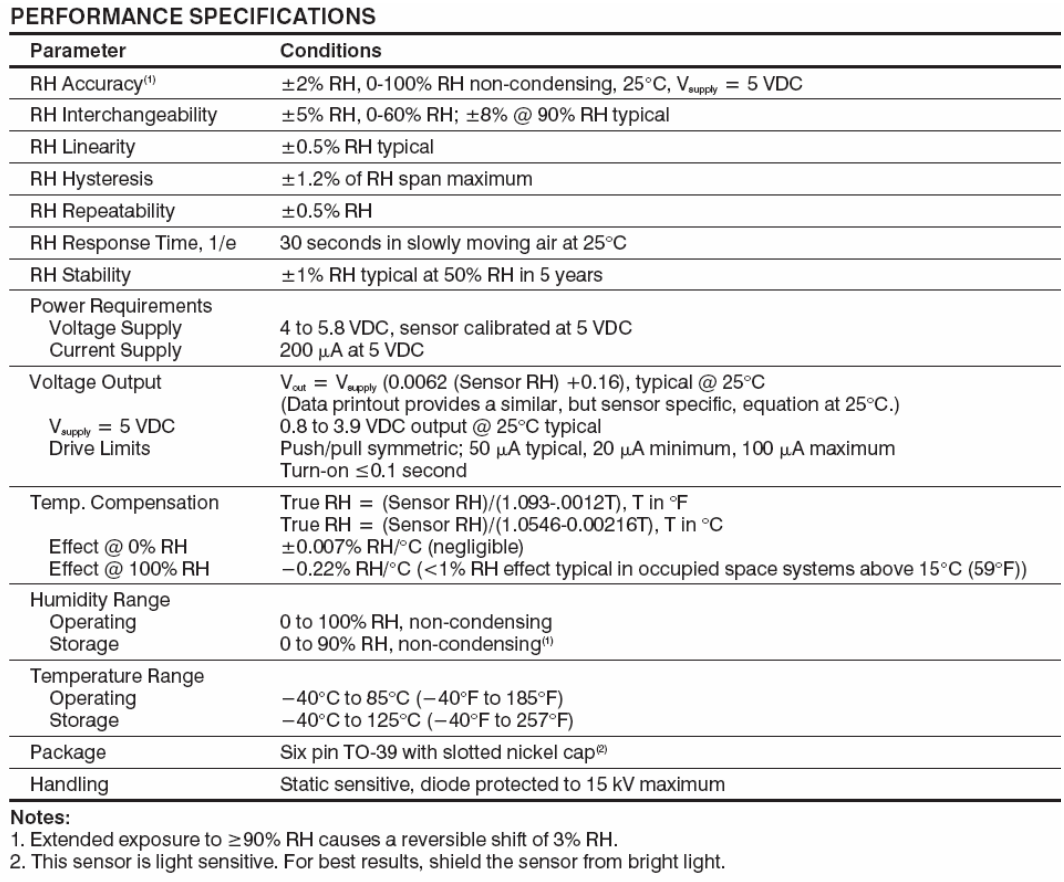


Figure 4 : Principle of Humidity measure by Honeywell ® sensors



PERFORMANCE SPECIFICATIONS

Parameter	Conditions
RH Accuracy[1]	±2% RH, 0-100% RH non-condensing, 25°C, V_{supply} = 5 VDC
RH Interchangeability	±5% RH, 0-60% RH; ±8% @ 90% RH typical
RH Linearity	±0.5% RH typical
RH Hysteresis	±1.2% of RH span maximum
RH Repeatability	±0.5% RH
RH Response Time, 1/e	30 seconds in slowly moving air at 25°C
RH Stability	±1% RH typical at 50% RH in 5 years
Power Requirements	
Voltage Supply	4 to 5.8 VDC, sensor calibrated at 5 VDC
Current Supply	200 µA at 5 VDC
Voltage Output	V_{out} = V_{supply} (0.0062 (Sensor RH) +0.16), typical @ 25°C (Data printout provides a similar, but sensor specific, equation at 25°C.)
V_{supply} = 5 VDC	0.8 to 3.9 VDC output @ 25°C typical
Drive Limits	Push/pull symmetric; 50 µA typical, 20 µA minimum, 100 µA maximum Turn-on ≤0.1 second
Temp. Compensation	True RH = (Sensor RH)/(1.093-.0012T), T in °F True RH = (Sensor RH)/(1.0546-0.00216T), T in °C
Effect @ 0% RH	±0.007% RH/°C (negligible)
Effect @ 100% RH	−0.22% RH/°C (<1% RH effect typical in occupied space systems above 15°C (59°F))
Humidity Range	
Operating	0 to 100% RH, non-condensing
Storage	0 to 90% RH, non-condensing[1]
Temperature Range	
Operating	−40°C to 85°C (−40°F to 185°F)
Storage	−40°C to 125°C (−40°F to 257°F)
Package	Six pin TO-39 with slotted nickel cap[2]
Handling	Static sensitive, diode protected to 15 kV maximum

Notes:
1. Extended exposure to ≥90% RH causes a reversible shift of 3% RH.
2. This sensor is light sensitive. For best results, shield the sensor from bright light.

Table 4 : Characteristics of some Honeywell ® humidity sensors

Regulation and control of H_R

The basic way to control humidity at inlet of a gas system is in fact to control water partial pressure in the system. In turns, this also leads to control temperature in system.

First, assume that there is no water condensation or evaporation into chamber.

Let T_0, p_{w0}, p_0 the set point values of temperature, water partial pressure and total pressure in the system. This corresponds to a humidity set point value H_{R0} :

$$H_{R0} = \frac{p_{w0}}{P_w^0(T_0)}$$

The control of humidity into chamber H_{R0} means to control both p_{w0} and temperature T_0. Let's suppose that the temperature T_0 into chamber is controlled by independent device, the control of H_{R0} leads to control the internal water vapour pressure p_{w0}.

Let $\boldsymbol{p}$ and $\boldsymbol{T}$ the total pressure and the temperature before the chamber. If water condensation in the circuit between the mass flow controller and the chamber is avoided, the water mole fraction remains identical whatever the total pressure, so that :

$$y_w = y_{w0} = \frac{p_w}{p} = \frac{p_{w0}}{p_0}$$

In order to obtain the set point value $\boldsymbol{p_{w0}}$, the water partial pressure in the entrance circuit must be :

$$\boldsymbol{p_w = p_{w0}}\ \frac{p}{p_0}$$

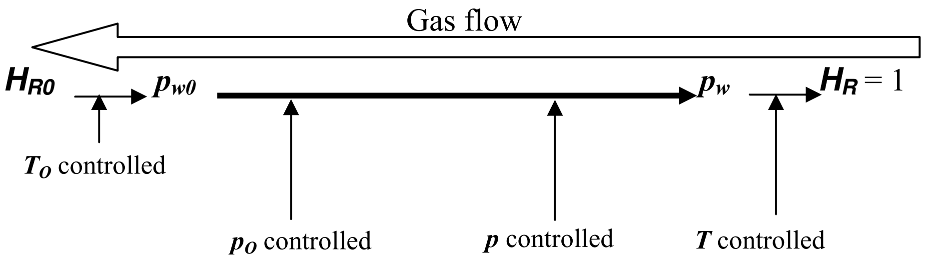
Therefore control of $\boldsymbol{p_{w0}}$ leads in fact to control $\boldsymbol{p_w}$. This can easily be performed by controlling the temperature $\boldsymbol{T}$ of a water bath at the entrance gas circuit, such as the gas is water saturated : $\boldsymbol{H_R} = 1$. This leads to :

$$\boldsymbol{p_w = P^0_w (T)}$$

Finally, the control of humidity $\boldsymbol{H_{R0}}$ into chamber is obtained by controlling, firstly the temperature into chamber and secondly the temperature of a water bath before the entrance in the chamber so that :

$$\boldsymbol{H_{R0}} = \frac{P^0_w(T)}{P^0_w(T_0)}\ \frac{p_0}{p}$$

This means that the following chain of controlled variables must be installed :



This means that the four variables $\boldsymbol{T_0}$, $\boldsymbol{p_0}$, $\boldsymbol{T}$ and $\boldsymbol{p}$ must be fixed and controlled for controlling $\boldsymbol{H_{R0}}$ which is always a difficult task. Generally $\boldsymbol{T_0}$ and $\boldsymbol{p_0}$ are fixed, considering other constraints than humidity. Therefore $\boldsymbol{T}$ and $\boldsymbol{p}$, the temperature and pressure of the water bath prior the entry of gas into chamber, are the only manipulated variables responsible of control of the variable $\boldsymbol{H_{R0}}$.

***Secondly, assume that there is water condensation or evaporation into chamber*.**

This is the more common case. The former analysis remains valid but H_{R0} must be considered as the water humidity of the incoming gas stream. The final water humidity into chamber depends on water exchange rate between gas and liquid into chamber. Formally the control strategy remains the same as previously (by controlling T and p) but the analytical relation relative humidity into chamber and T, p, T_0, p_0 is no longer valid and must include a net exchange rate term R_{H2O}.

4 ASSESSMENT OF GAS COMPOSITIONS : SELECTION OF INSTRUMENTATION

4.1 Composition variables

By definition all composition variables are intensive properties. They are often reported as volumic percentages (% v or ppmv). Considering the ideal gas law applies (with high content of CO_2 this is not completely true and may introduce 1 to 3 % error in the conversion depending on temperature and pressure), the volumic percentages are equal to molar fractions. In any case, it seems preferable to work with gas partial pressures which are directly useful for understanding physical and gas liquid equilibria. The components partial pressures to assess are :

- CO_2
- CH_4
- C_2H_4
- H_2O
- H_2
- NH_3
- H_2S
- VFA
- Trace gases : organic volatiles, N_2, NO_x ...etc.

Among these components, only the water content in the gas phase can be modified between the chamber and gas analysis device, due to mandatory condensation unit or the outlet circuit. Condensing the water of the gas phase may have a slight impact on the composition of the gas phase. Indeed, part of the water-soluble compounds is dissolved together with water.

These different variables are linked by classical thermodynamic laws. At a first approximation, ideal gas law and Dalton law (total pressure equals the sum of the partial pressures) are considered valid. More sophisticated thermodynamic models (equations of state such as Van der Waals, Redlich Kwong...etc. and non ideal gas mixing rules) can be used for further studies. These models are classically handled by commercial process simulation softwares. Presently, it can be considered that such sophistication will bring corrective terms (*ca* 1 to 3 %) but will not explain the major variations which are under investigation.

4.2 Outlet gas circuit

In addition to pressure regulation purposes as schemed in Figure 2, the outlet gas circuit has the following functions :

- gas pressure regulation inside the chamber (zero order control of chamber pressure) ;

- interface with gas composition measurement units (control of pressure, temperature, humidity before analysers) ;
- reduction (or control of humidity) of water partial pressure between chamber and other gas circuits ;
- sanitation of gas stream before other compartments.

There are several possibilities for adding to the scheme reported in Figure 2 a filtering unit, a dehumidifier and a gas sampling tube. The final best assembly depends on specifications regarding net gas flow, humidity in chamber and sanitation constraints, knowing that drying of gas before analysis and preventing any condensation in the gas circuit is probably the most constraining thing.

An example of engineering of outlet gas circuit for compartment 1 of MELiSSA is given in TN 74.3. This kind of study must be done in each specific case.

4.3 Gas-specific techniques for assessment of gas composition

There are reliable specific techniques for assessment of CO_2 and O_2 partial pressures (or volumic percentages). This has been reviewed many times. For CO_2, the basic technique uses infrared (IR) analysis. For O_2, several techniques exist. Most apparatus for oxygen analysis are based on paramagnetic measurement, electrochemical analysis or zirconium technology.

4.3.1 GAS-SPECIFIC TECHNIQUES FOR ASSESSMENT OF CO_2

Photometric CO_2 gas analyzer

Carbon dioxide absorbs infrared light. This property is used in photometric gas analyzers where gas is flowing through a photometric cell where the amount of absorption is measured. This kind of apparatus is robust. Accuracy of this kind of apparatus is generally 1 % of full scale. It is possible to find analyzers able to measure carbon dioxide in several range (0 - 1%, 0 - 5%, 0 - 10%...).

CO_2 measurements with semiconductors

For measuring carbon dioxide, various sensor principles are being investigated on the basis of solid state electrolysis and semi-conductors as is shown in Figure 5. Nano-sized titanate- or stanate-ceramics or ion conductors such as NASICON (**Na**trium **S**uper **I**onic **Con**ductor) could be used. While NASICON allows for a three dimensional conductivity of sodium through its complex lattice, titanate or stanate changes its resistance as the CO_2 concentrations change.

Semiconductors react directly to changes in the CO_2 portion. The conductivity of the semiconductor material is influenced through an accumulation of molecules in the lattice. The simple determination of the resistance makes it possible to measure the gas portion. Sensors based on solid state electrolysis generally make it possible to achieve a faster response time. The functional principles in use here is the varying reaction mechanisms on the diagnostic and reference electrode.

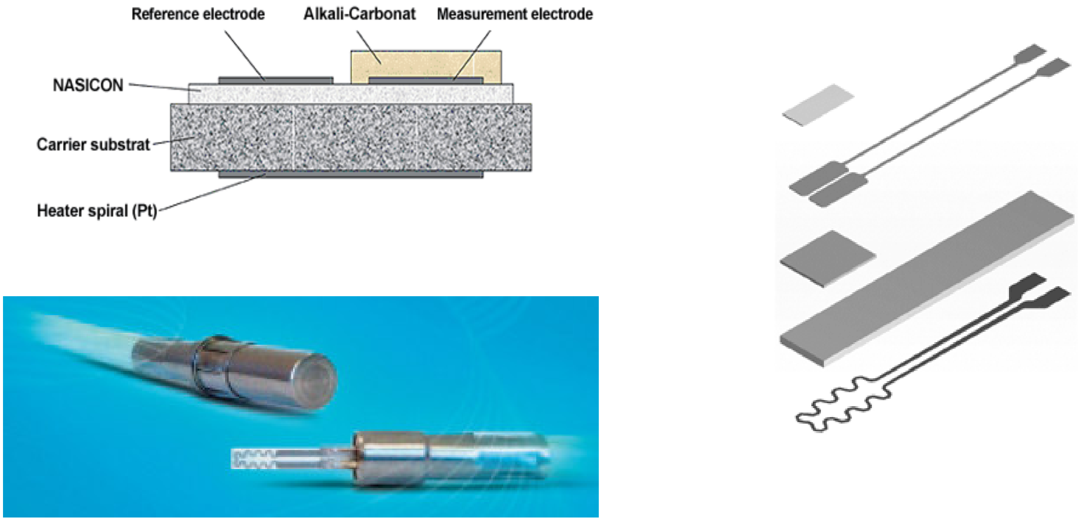


Figure 5 : Semiconductor carbon dioxide sensor

4.3.2 GAS-SPECIFIC TECHNIQUES FOR ASSESSMENT OF O_2

Paramagnetic oxygen analysis

The paramagnetic measurement is based on the attraction of oxygen towards a magnetic field. The principle of the apparatus is shown in Figure 6.

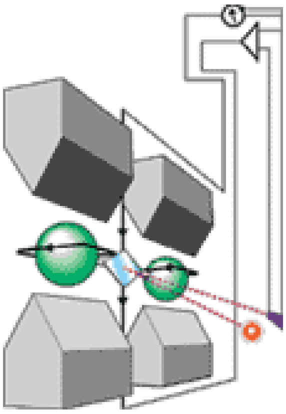


Figure 6 : Principle of the paramagnetic measurement system

The oxygen is attracted toward a magnetic field and influences the positioning system of a mirror that is located in the field. The change in the mirror position is detected by photocells and correlated to the oxygen gas concentration. Notice that the positioning system of this kind of apparatus is fragile and susceptible to mechanical disturbance. The measurement range is between 0 and 100 vol % of oxygen with an accuracy of 0.01 %. The principle in an industrial design is not shock proof. Custom made modifications will be necessary for a space version of this kind of apparatus especially a “stabilizing” device to prevent failure due to shocks during launch.

Electrochemical analysis

The electrochemical sensor contains an electrolyte. A PTFE membrane is provided to let O_2 molecules into the sensor. At a metal electrode, the O_2 molecules are chemically transformed. This chemical reaction produces an electrical current that is measured. This sensor can measure in a range of 0 - 100 % with an accuracy of 0.05 to 0.1 %. The disadvantage is the fact that the sensor is filled with fluid electrolyte. Some types use a solid electrolyte, but the accuracy is about 1 %, which is too high for the application in the BioRat project.

Zirconia oxygen analysis

Zirconia is a ceramic that conducts electricity at high temperature by the movement of oxygen ions. This property is used in oxygen measuring cell for applications such as combustion gas analysis or gas purity measurement. Figure 7 shows the concept of the sensor. Due to the difference in oxygen concentration between the two sides of a zirconia disk, a voltage is generated and detected by the electrodes. Zirconia sensor needs a high operation temperature (300°C) and the optimal range for measurement is between 0 - 21 vol % of oxygen, with an accuracy of 0.01 %.

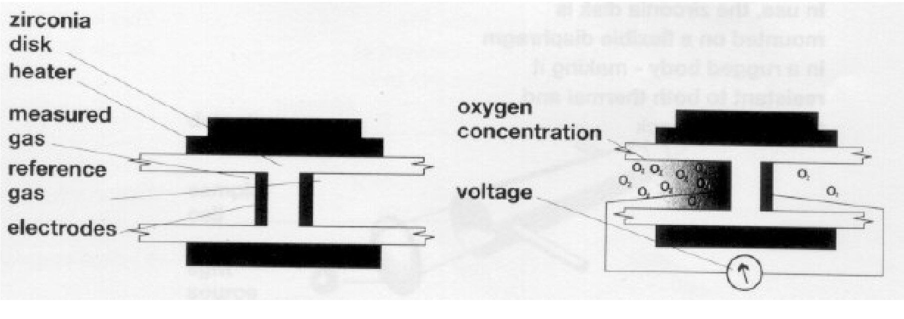


Figure 7 : Principle of Zirconia oxygen analyzer

Fluorescence

Other possibility is a fluorescence based sensor. The principle of fluorescence sensors uses the emission characteristics of specifically sensitive dyes as it is presented in Figure 8. The dyes' fluorescence behaviour depends on the concentration of the analyte towards which they react sensitively. Oxygen is a typical fluorescence quencher. Nowadays, measuring oxygen concentrations is one of the key applications in fluorescence sensor technology.

Short pulses of blue LED light are transmitted along an optic fiber probe to excite a platinum-based fluorophore situated at the probe tip. The fluorophore is permanently immobilised and enclosed within a silicone matrix. The resulting emission of fluorescent light, quenched by the presence of oxygen molecules, travels back up the fibre and is detected by the instrument. The lifetime of fluorescence is inversely proportional to the concentration of oxygen.

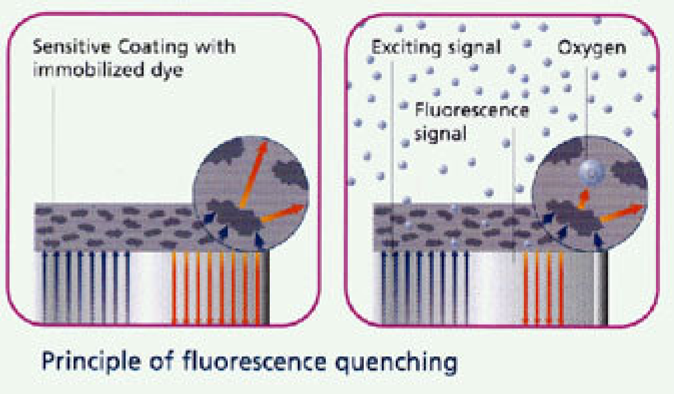


Figure 8 : Principle of fluorescence quenching

Fluorescence lifetime is longest at low pO_2, making probes more sensitive in the physiological range 0 - 60 mmHg. Also, as the measurement is based on fluorescence lifetime rather than fluorescent intensity it is much less prone to artefacts (e.g. due to variation in the intensity of the light source, ambient lighting, or photo-bleaching etc.).

Compensation for the effects of temperature is required since fluorescent lifetimes are affected by changes in temperature. Temperature is measured by a fully integrated thermocouple, allowing simultaneous monitoring of pO_2 and temperature as well as automatic temperature correction.

This kind of sensor has the advantage of having a low drift calibration (> 3 months) and a good response time (around 10 seconds for a gaseous media).

Drift

Oxygen sensors have the tendency of drifting. For a paramagnetic based apparatus, the drift is about 0.01 % to 0.1 % of oxygen per week. This means that an auto calibration unit may be needed including reference gasses, valve for gas routing and a controller unit. Oxygen apparatus need accurate control of the flow, pressure and humidity of the sample that is passing through the analyzer.

In general, CO_2 sensors are more robust. The sensor can easily be combined with the peripheral equipment already needed for the O_2 sensor. The measurement cell is relatively small.

4.4 Non gas-specific technique for assessment of gas composition : GC/MS

A complete review of non specific techniques for assessing gas compositions has been done in TN 74.3 which concerns the characterisation of gas effluent from compartment I.

The presentation will not be given here but all the results obtained can easily be applied for characterization of gas effluent from metabolic chambers. In this case, the basic composition is not far from air composition, knowing that trace gas (which are often important to be characterised) are VFA, NH_3, H_2S and ethylene for higher plant chamber.

The main outputs of the previous study are summarized as follows.

4.4.1 GAS COMPOSITION ASSESSMENT : GAS CHROMATOGRAPHY

Gas chromatography is used to separate volatile organic and inorganic gas mixtures. A gas chromatographic analysis is based on the sample components being partitioned between the gas (mobile phase) and a liquid or solid adsorbent (stationary phase) by passing through a separative column. Partition of the sample components depends on column operating parameters, including carrier gas flow rate, temperature, vapor pressure and on properties of the liquid or solid adsorbent. A sample is introduced through the injection device. It is carried through the column by an inert gas flowing at a controlled rate. Due to different retention (or adsorption properties into the column) of the gas components in the mixture, the components of the gas flow exit the column separately.

The **carrier gas** must be sufficiently pure (minimum 99.995%) and inert with respect to the stationary phase. Helium is currently used. Traces of unwanted contaminants in the carrier gas must be removed by means of molecular sieves scrubbers, filters and filter-dyers. These devices must be inserted in the supply line as close as possible to the gas chromatographic column as practical.

There exist different types of **injection device**. In the case of the gas effluent of a metabolic chamber, it can be clearly shown that the major components (CO_2, N_2 and O_2) will be assessed with a split injection with an inert gas (He or Ar) as carrier. The minor compounds will be assessed with a splitless device provided that the column will be able to separate H_2, NH_3 and VFA and that the major gas compounds will not poisoned the detection. The trace compounds can be assessed with an enrichment system, provided that the other major compounds will not compete too much to fixation onto the solid adsorbent with the target components (aroma, VFA...).

There exists two types of **columns** : packed columns and capillary (open tubular) columns. The packed columns are used to separate permanent gas like CO_2 - H_2 - N_2 - O_2 - CH_4…etc.. The capillary columns (open tubular) are used to separate polar or non polar compounds in function of the stationary phase. The system is adapted to Volatile Fatty Acids. The detector can be respectively **thermal conductibility detector** and **flame ionisation detecto**r according to the column is a packed or capillary column. These detectors are economic and universal but they don't give any information about the nature of detected substances. Calibration of detector and column assembly re necessary, knowing that significant deviations may occur during long (several days) operations.

4.4.2 MASS SPECTROMETRY (MS)

Basically the mass spectrometer is a partial pressure analyzer of compounds carried in a gas phase. It is conceived to determine the residual gases in the secondary vacuum apparatus. It can detect the component of a gas mixture :

- qualitatively with the mass numbers ;
- quantitatively with a disconvolution procedure.

A mass spectrometer is composed by three parts : the ions source, the ion separator and the ion detector.

Several kinds of **ions sources** are used. The electronic bombardment source (electron impact ionization) is currently used because it can give stable operation over long period time. The others (surface ionization source, sparks source, photo-ionization or laser source and field emission source) are eliminated because they cannot ionize all the gas or they are less sensitive.

The **ion separation** is the characteristic element of a gas analyzer and its performances determine the performances of the apparatus. The system separates in time or in space the ions in function of the ratio mass / charge of each ion (m/z ratio). There exist four types of ions separators :

- Time of flight separator (TOF separator).
- Magnetic field
- Fourier transform mass separator

- Quadripole mass separator

Quadripole instruments can be made very compact (which is of interest for space applications) and sensitive. This is already used for spacecraft atmosphere characterisation. These instruments are ideally suited for very fast selected ion monitoring and multiple ion detection.

For the analysis of gases, a capillary inlet must be chosen method in order to reduce the gas pressure form the sample pressure to ion chamber pressure. The pressure is firstly reduced from 300 kPa to approximately 100 Pa using a capillary. The necessary pressure drop is created by a rotary pump. Under this condition flow in the capillary will be laminar. Flow in the connection line to the rotary pump also has to be in the laminar flow region. This avoids enrichment of compounds according to their masses, which only occurs in the molecular flow region where the mean free path of molecules is larger than the inner diameter of the tube. At temperature below the boiling point of the volatile compounds, condensation may occur if the vapor pressure is sufficiently low. It is necessary to heat the capillary and to use inert materials.

4.4.3 QUALITATIVE AND QUANTITATIVE ANALYSIS

The qualitative analysis consists in identifying the peaks (intensity in function of the m/z ratio). Every chemical compound being decomposed with a defined stoichiometry into a series of ions, a MS spectrum can be interpreted as a fingerprint, specific of each chemical compound. This leads to use MS to determine the chemical composition of a gas in the mass spectrometer chamber. The intensity of a peak is directly proportional to the ionic concentration in the ionization chamber for all ions having the same m/z ratio. The ionic concentration is also directly proportional to the partial pressure of the incoming gas in the source. If several ions have the same ration m/z but if they have a different nature, only one peak is observed, but the intensity of this peak is proportional to the sum of the partial pressure. The total pressure is taken from the total pressure plate mounted in the ion source of the sensor. A more accurate total pressure measurement can be made with an ion gauge.

The peak height is representative of the partial pressure of a component and depends on the parameters such as the ionization probability, the fragmentation factor, a transmission factor and the sensitivity of the detector. An algorithm is used to determine the partial pressure specific to each gas. The formula for determining the partial pressure of gas A is based upon peak at mass B and is given by multilinear regression analysis.

4.4.4 GC/MS ANALYSIS

From the above, it clearly appears that neither GC nor MS alone can solve the problem of on-line analysis of the gas phase effluent of any metabolic chamber. The best solution is to use, from one hand the separation capacities of a chromatograph and the general capacities of MS for identifying and quantifying gas compounds, even if they are present in gas mixtures. Gas chromatography coupled to mass spectrometry is a method that combines the features of gas chromatography and mass spectrometry to identify different substances.

For a metabolic chamber, the exhaust gas contains permanent gases (O_2, N_2, CO_2, H_2, C_2H_4, H_2O, …) and Volatile Fatty Acids. Considering that the simultaneous quantification of VFA and permanent gas will be hardly achievable simultaneously because of the difference in concentrations, the analyzer has to be composed of two gas chromatographic columns : one with a packed column for permanent gases separation and one with a capillary column for VFA. This is schemed in Figure 9.

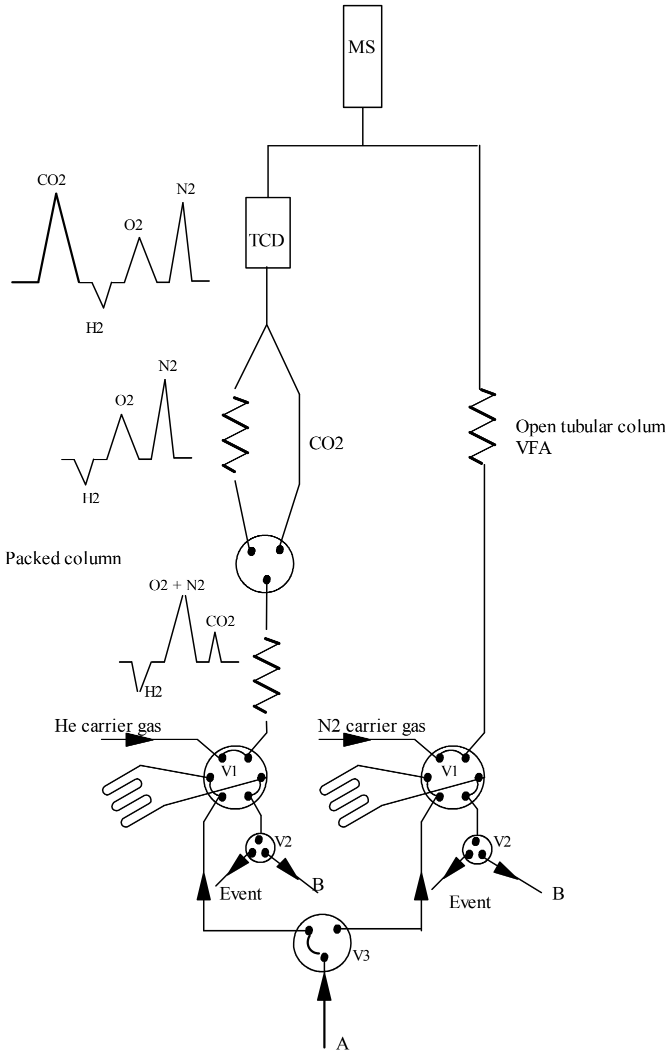


Figure 9: Two ways gas chromatograph circuit

The gas is alternatively distributed in the packed column and the capillary column with the V3 valve. It is important not to inject carrier gas (He or N_2) in the outlet gas circuit, so design the two ways gas chromatograph circuit with two events. Analysis is performed in 3 steps to avoid pollution with vector gas. The three steps are :

- Step 1 : Gas sample is introduced in the loop to rinse the loop. Gas return in the outlet gas circuit in B.
- Step 2 : Carrier gas is introduced during 12 s. the vector gas pushes the compartment I gas present in the loop toward the separation column. Analyze can be possible
- Step 3 : Gas sample is introduced in the loop to purge vector gas. To prevent pollution of the gas circuit, this gas is ejected by event. The purge time is estimated at time necessary to pass 5 loop volumes.

The interface between the gas chromatograph and the mass spectrometer is a separator that preferentially removes the gas chromatograph carrier and permits a flow of enriched gas sample to enter the spectrometer. For the permanent gas, a TCD detector detects the peaks in order to switch the gas sample (after column separation) for entering the spectrometer. Generally, there is a molecular jet separator between GC and MS. Direct coupling to the mass spectrometer is usually used for capillary columns. As the outlet gas flow of the fermentor, the circuit to the Mass Spectrometer analyzer must be heated to at least 40 - 65°C for avoiding any condensation. Water remains a problem either for the detection system or for the chromatographic columns. The choice of water trap depends of the gaseous phase. For example, silica gel adsorbs CO_2 and others can adsorb VFA. But to protect the analyzer, it is necessary to have a dew-point of - 40°C at the entrance of the gas analysis system.

5 SENSITIVITY ANALYSIS FOR RATES ESTIMATION

5.1 Steady state

The objective of this section is to focus on numerical sensitivity analysis for rates estimation ; firstly, this is done for steady state assumptions, *i.e.* 0, 1st , 2nd order models as defined in Table 3.

The departure equations are Eqs. 16 and 17 leading to calculate R_{O2} and R_{CO2} in the simplest model :

$$R_{O2} = \phi_n^{(1)} \, [\alpha \, y_{O2}^{(2)} - y_{O2}^{(1)}] \qquad \text{(Eq. 16)}$$

$$R_{CO2} = \phi_n^{(1)} \, [\alpha \, y_{CO2}^{(2)} - y_{CO2}^{(1)}] \qquad \text{(Eq. 17)}$$

where α represents the ratio $\phi_n^{(2)} / \phi_n^{(1)}$ of molar flows at outlet and inlet of chamber.

$$\alpha = \frac{\phi_n^{(2)}}{\phi_n^{(1)}} = \frac{y_{inert}^{(1)}}{y_{inert}^{(2)}} \qquad \text{(Eq. 33)}$$

The last equation is obtained by writing the mass conservation of inert between input and output. The inert gas should be Nitrogen or Argon and the mole fractions of inert can be either measured directly by GC/MS as described above or calculated with sum equations (Eqs. 15a, 15b) knowing the other mole fractions which change between inlet and outlet.

Whatever the measurement technique used the purpose of this section is to give an insight into reliability of calculated variables in the context of actual accuracy of measured variables.

From a general point, let the variable y be given as a function of several measured variables $x_1, x_2 \ldots$: $y = f(x_1, x_2 \ldots)$. The relative error $\Delta y/y$ is obtained from the differential of $Ln\, f$, so that :

$$d\,(Ln\, y) = \frac{dy}{y}$$

$$d\,(Ln\, f) = \frac{1}{f} \, [\left| \frac{\partial f}{\partial x_1} \right| dx_1 + \left| \frac{\partial f}{\partial x_2} \right| dx_2 + \ldots]$$

So that :

$$\frac{\Delta y}{y} = \frac{1}{f} \Big[\left| \frac{\partial f}{\partial x_1} \right| \Delta x_1 + \left| \frac{\partial f}{\partial x_2} \right| \Delta x_2 + \ldots \Big]$$

When applied to Eq. 16, this leads to :

$$\frac{\Delta R_{O2}}{R_{O2}} = \frac{\Delta \phi_n^{(1)}}{\phi_n^{(1)}} + \frac{y_{O2}^{(2)}\,\Delta\alpha + \alpha\,\Delta y_{O2}^{(2)} + \Delta y_{O2}^{(1)}}{\alpha\, y_{O2}^{(2)} - y_{O2}^{(1)}} \qquad \text{(Eq. 34)}$$

and similarly for CO_2 :

$$\frac{\Delta R_{CO2}}{R_{CO2}} = \frac{\Delta \phi_n^{(1)}}{\phi_n^{(1)}} + \frac{y_{OC2}^{(2)}\,\Delta\alpha + \alpha\,\Delta y_{CO2}^{(2)} + \Delta y_{CO2}^{(1)}}{\alpha\, y_{CO2}^{(2)} - y_{CO2}^{(1)}} \qquad \text{(Eq. 35)}$$

From Eq. 33, we obtain :

$$\frac{\Delta\alpha}{\alpha} = \frac{\Delta y_{inert}^{(1)}}{y_{inert}^{(1)}} + \frac{\Delta y_{inert}^{(2)}}{y_{inert}^{(2)}} \qquad \text{(Eq. 36)}$$

In order to give an order of magnitude of the resulting error $\frac{\Delta R_{O2}}{R_{O2}}$, let us give values to the variables :

Δy_{O2} = 0.1 % = 1 000 ppm = ± 500 ppm = ± 0.0005 = ± 0.05 %

$\boldsymbol{y}_{O2}^{(1)} = 0.2093$ $\qquad \frac{\Delta y_{O2}^{(1)}}{y_{O2}^{(1)}} = 0.1$ % $\qquad$ (value for dried air with a paramagnetic analyser)

$\boldsymbol{y}_{O2}^{(2)} = 0.2193$ $\qquad \frac{\Delta y_{O2}^{(2)}}{y_{O2}^{(2)}} = 0.1$ % $\qquad$ (slight increase of oxygen due to photosynthesis)

$\boldsymbol{\alpha} = 1$ $\qquad \frac{\Delta\alpha}{\alpha} = 0.001$ $\qquad$ (No significant change of incoming and outcoming gas flow).

Assuming no error in measurement of gas flow $\phi_n^{(1)}$, the result of calculation with previous Eq. 34 leads to :

$$\frac{\Delta R_{O2}}{R_{O2}} = \frac{0.209 * 0.001 + 0.21 * 0.001 + 0.21 * 0.001}{0.01} = 3 * 0.2 * 0.1$$

$$\frac{\Delta R_{O2}}{R_{O2}} = 0.06 = 6\ \%$$

This shows that when measuring oxygen mole fraction with good accuracy at 0.1 %, ($y_{O2} = \bar{y}_{O2} \pm 0.0005$) the final result for R_{O2} is obtained with no more than 5 % accuracy. This requires, from one hand to have a very reliable measurement technique, and secondly to be in position that the approximations in estimating the other variables (such as α or the water partial pressure at the measurement point) are satisfied. This is not a simple task and this must be carefully examined before classifying and selection of suitable instrumentation and implementation in a gas circuit.

The results for R_{CO2} error estimation (Eq. 35) are similar. Let's give the following values :

Δy_{CO2} = 50 ppm = 0.005 % = ± 25 ppm

$y^{(1)}_{CO2} = 0.5\ \% = 5\ 000$ ppm $\qquad \dfrac{\Delta y^{(1)}_{CO2}}{y^{(1)}_{CO2}} = 1\ \%$

$y^{(2)}_{CO2} = 0.05\ \% = 500$ ppm $\qquad \dfrac{\Delta y^{(2)}_{CO2}}{y^{(2)}_{CO2}} = 10\ \%$

$$\frac{\Delta R_{CO2}}{R_{CO2}} = \frac{1}{0.5 - 0.05}\,(0.05 * 0.01 + 0.05 * 0.1 + 0.01 * 0.5)$$

$$= 2.\ 10^{-2} = 2\ \%$$

This leads to consider as a rule of thumb that even with less accuracy for CO_2 mole fraction measurement (here Δy_{CO2} = 50 ppm = ± 25 ppm), the resulting error amplification for R_{CO2} estimation is less dramatic than for oxygen, leading here to an estimated error of 2 %. It can also be sown that a slight error in flow difference (characterised here by α) will not impair dominantly the overall accuracy of the results.

Nevertheless, as previously for oxygen, it is important to correctly choose instrumentation and implementation on gas circuit for measuring CO_2 partial pressure (or CO_2 mole fraction) with the aim of calculating CO_2 reaction rate into a metabolic chamber.

Whatever the case, before any final choice of instrumentation, the error on the exchange rates of O_2, CO_2 and eventually other gas must be estimated using Eqs. 34 and 35 in order to get an idea of the accuracy of the results. This is even more important when these calculated variables are further used for process control purposes.

5.2 Transient state

For the moment, the error estimation has been done with the simplest model (zero-order model) for gas phase. The previous error estimation must be done with models of higher complexity, including liquid mass balance and accumulation terms. This is described by fourth-order model at steady state (Eq. 28) and fifth-order model in transient state (Eqs. 31 and 52) :

$$R_{ni} = \phi_n^{(1)} [\frac{y_{inert}^{(1)}}{y_{inert}^{(2)}} y_i^{(2)} - y_i^{(1)}] + \phi_L^{(2)} c_i^{(2)} - \phi_L^{(1)} c_i^{(1)} \qquad \text{(Eq. 28)}$$

Gas phase

$$\frac{d}{dt} (y_i \frac{pV_G}{RT}) = \phi_n^{(1)} y_i^{(1)} - \phi_n^{(2)} y_i^{(2)} + K_{Li}\, a\, (c_i - c_i^*)\, V_L \qquad \text{(Eq. 31)}$$

Liquid phase

$$\frac{d}{dt} (V_L c_i) = \phi_L^{(1)} c_i^{(1)} - \phi_L^{(2)} c_i^{(2)} + K_{Li}\, a\, (c_i^* - c_i)\, V_L + R_{ni} \qquad \text{(Eq. 32)}$$

The error estimation analysis presented in the previous section remains valid for evaluating the error on the difference $\phi_n^{(1)} y_i^{(1)} - \phi_n^{(2)} y_i^{(2)}$. In order to obtain error estimation on R_{ni}, it is necessary to add the error estimation related to convective flux transported by liquid flow $\phi_L^{(1)} c_i^{(1)} - \phi_L^{(2)} c_i^{(2)}$ and the influence of gas phase and liquid phase accumulation terms $\frac{pV_G}{RT} \frac{dy_i}{dt}$ and $V_L \frac{dc_i}{dt}$ respectively.

The error estimation is not simple to perform in the general case. Assuming no time change of gas volume, the residence times of both gas and liquid phase can be calculated :

$$\tau_G = \frac{pV_G}{RT\phi_n^{(1)}} \qquad \text{(Eq. 37)}$$

$$\tau_L = \frac{V_L}{\phi_L^{(1)}} \qquad \text{(Eq. 38)}$$

Eqs. 31 and 32 are then rewritten as follows :

$$\frac{K_{Li}a}{\phi_n^{(1)}} \boldsymbol{(c_i - c_i^*) V_L = \alpha\ y_i^{(2)} - y_i^{(1)} + \tau_G} \frac{dy_i^{(2)}}{dt} \qquad \text{(Eq. 39)}$$

$$\boldsymbol{R_{ni}} = K_{Li}a\ \boldsymbol{(c_i - c_i^*) V_L} + \phi_L^{(1)}\ \boldsymbol{[\beta\ c_i^{(2)} - c_i^{(1)} + \tau_L} \frac{dc_i^{(2)}}{dt}\boldsymbol{]} \qquad \text{(Eq. 40)}$$

Combining the previous Eqs. 39 and 40, the instantaneous reaction rate for compound ***i*** (***Rni***) is written :

$$\boldsymbol{R_{ni}} = \phi_n^{(1)}\ \boldsymbol{(\alpha\ y_i^{(2)} - y_i^{(1)} + \tau_G} \frac{dy_i^{(2)}}{dt}\boldsymbol{)} + \phi_L^{(1)}\ \boldsymbol{(\beta\ c_i^{(2)} - c_i^{(1)} + \tau_L} \frac{dc_i^{(2)}}{dt}\boldsymbol{)} \qquad \text{(Eq. 41)}$$

with α and β given by :

$$\boldsymbol{\alpha} = \frac{\phi_n^{(2)}}{\phi_n^{(1)}} \qquad \text{(Eq. 33)}$$

$$\boldsymbol{\beta} = \frac{\phi_L^{(2)}}{\phi_L^{(1)}} \qquad \text{(Eq. 42)}$$

The error on the calculation of instantaneous reaction rate for compound ***i***, $\frac{\Delta R_{ni}}{R_{ni}}$ is therefore formed of the error on the term $\phi_n^{(1)}\ \boldsymbol{(\alpha\, y_i^{(2)} - y_i^{(1)})}$ plus the error on the term $\phi_L^{(1)}\ (\boldsymbol{\beta\, c_i^{(2)}} - \boldsymbol{c_i^{(1)}})$ plus the error on the accumulation terms $\boldsymbol{\tau_G} \frac{dy_i^{(2)}}{dt}$ and $\boldsymbol{\tau_L} \frac{dc_i^{(2)}}{dt}$**.**

1 - The estimation of error on the term $\phi_n^{(1)}\ \boldsymbol{(\alpha\, y_i^{(2)} - y_i^{(1)})}$ has been presented in the previous section. The conclusions completely apply with the new set of assumptions of the more evolved models.

2 - Generally, the error on the liquid phase convective term $\phi_L^{(1)}\ \boldsymbol{(\beta\ c_i^{(2)} - c_i^{(1)})}$ can be neglected because the liquid phase concentrations remains low. This is true for permanent gas such as oxygen, nitrogen, C_2H_4...etc. This must be more deeply studied for other gas such as VFA and CO_2, the value of liquid phase concentrations strongly depending on pH. In some cases, when pH values are high, the transported amounts are much higher in liquid phase than in gas phase. In such cases, error calculation must be carefully performed from *a priori* estimations in order to evaluate eventual error amplification by taking a difference between inlet and outlet concentrations in liquid phase. The conclusions for liquid phase are similar to the developments for the gas phase (previous section), the main result being that the calculations must be done in each specific case.

3 - The error on accumulation terms $\tau_G \dfrac{dy_i^{(2)}}{dt}$ and $\tau_L \dfrac{dc_i^{(2)}}{dt}$ is easily estimated knowing the residence times. We present the calculation for the gas phase. By example, coming back to the case of oxygen with the values considered in previous section and with a gas residence time of 10 min and mole fraction variation of 0.05 % min^{-1}, we have :

$$\tau_G \frac{dy_{O2}^{(2)}}{dt} = 0.5\ \% = 5.10^{-3}$$

If this term is neglected in R_{O2} calculation, the error on R_{O2} is then :

$$\frac{\Delta R_{O2}}{R_{O2}} = 0.06 + \frac{5.10^{-3}}{0.01} = 0.56$$

In other words, this means that the error on R_{O2} is more than 50 % if the gas accumulation term is neglected. Therefore for zero to third order models, it is easily calculated that the approximation on rates estimation linked to neglecting accumulation terms may lead to very serious errors.

It must be understood that the situation is completely different in the case of a bioreactors where the gas residence time is generally no more than few seconds. This means that for a metabolic chamber, the gas accumulation cannot be neglected or at least must be averaged over "long" periods of time.

This means that if the accumulation cannot be properly estimated, it is necessary : *(i)* to record values of gas mole fractions during 3 to 5 times the gas residence time, *(ii)* to take the mean and the variance and *(iii)* to perform a statistical analysis to verify if there is or not a systematic drift characteristic of a time-course evolution. If such an evolution of gas mole fraction is detected, the gas accumulation term must be calculated and taken into account for reaction rates evaluation. The result in terms of R_{ni} is therefore an averaged value over 3 - 5 times the gas residence time.

An other technique is a numerical estimation of time-course change of $y_i^{(2)}$, *i.e.* of the derivative $\dfrac{dy_i^{(2)}}{dt}$, and a calculation of instantaneous reaction rate R_{ni}, the results being eventually averaged over time.

Whatever the technique used, it clearly appears that careful data treatment must be done from gas balance measurements in order to assess the reaction rates. It also must be reminded that gas balance techniques for metabolic chambers are powerful tools but that the extrapolation from gas balances performed in bioreactors is not straightforward and must be studied in details. The main reasons are that errors amplification for rates evolutions may be even more dramatic in the case of metabolic chambers than in the case of reactors.

Nevertheless with an assumption of homogeneity of the gas phase, the general model as sketched by Eq. 41 (fifth-order model) is not formally more complex than a steady-state model, except that the data must be correctly treated and interpreted.

6 INFLUENCE OF GAS PHASE HETEROGENEITY

The previous calculations and error estimation have been performed considering homogeneously mixed gas phase with no pressure, temperature or mole fractions gradients.

It is obvious that such an assumption is far to be exact when metabolic chambers are several hundreds liters in volume. As a matter of proof, it is well known that the temperature gradients may reach 2 - 5 °C within a 3 m high chamber ; in the same way, mole fractions differences between floor and ceiling may reach several percents for heavy gas (CO_2, VFA).

It must be outlined however that the general form of mass balance equations still applied when the gas phase is not homogeneous. However, the measured variables in the outlet stream are no longer representative of mean values throughout the chamber.

Therefore, the general equation for calculating the reaction rate $\boldsymbol{R_{ni}}$ (Eq. 41) still applies but the gas accumulation term must be replaced as follows :

$$\boldsymbol{\tau_G}\,\frac{dy_i^{(2)}}{dt} \qquad\qquad \sum_j \tau_{Gj}\,\frac{dy_{ij}}{dt}$$

where $\boldsymbol{\tau_{Gj}}$ is the gas residence time in cell $\boldsymbol{j}$, the total gas volume having been divided into a series of cells. In each cell, the mole fraction of compound is $\boldsymbol{y_{ij}}$.

Of course, even not impossible, such a description gives much more complex modelling of the entire gas volume, introducing a multiplicity of state variables ($\boldsymbol{p_j}$, $\boldsymbol{T_j}$, $\boldsymbol{V_{Gj}}$, $\boldsymbol{y_{ij}}$) for all cells.

In order to solve this kind of model, it is necessary to write mass and energy balances between each cell and to add assumptions regarding convective terms between each cell. This kind of thing must be done using CFD softwares in order to properly define the cells and characterize state variables heterogeneity inside the chamber.

Although there is not a general gridding technique, some basic rules apply :

- Solid-gas interfaces must be properly considered as a special "boundary layer" cell where almost all reaction terms are generated ; that is also the cell where mass transfer rate controlling process may occur ;
- Bottom and top spaces of the chamber must be considered ;
- Mechanical and thermal balances between the cells must be written in order to decrease the global uncertainty of the system ;

Fluid mechanisms lead to compute the gas flow rate between the cells and the pressure gradients. Shear stresses field is also an output of this kind of simulation softwares

7 APPICATION TO METABOLIC CHAMBERS, *i.e.* CREW COMPARTMENT DESIGN

Most of the comments presented in this section are issued from a review published by one of us in 1997 in Journal of Theoretical Biology (Ortigues *et al.*, 1997). Although, the study concerns the comparison of various methods for calculating the instantaneous respiratory gaseous exchanges from discrete measurements in respiration chambers and is illustrated for animal respiration chambers, the results are directly applicable to crew compartment design and the characterisation of its behavior.

Whole body respiration chambers are characterised by a relatively low turnover of air (see above) which is known to be a handicap for measuring the energy expenditure associated with physiological phenomena of short duration. The difficulties encountered for evaluating the energy expenditure from inlet – outlet data are in fact the same when this knowledge must be used for a control and regulation strategy of a closed system where human activities of variable intensity are consuming oxygen and releasing CO_2. The study mentioned above (Ortigues *et al.*, 1997) shows the importance of the mathematical treatment and of the measurement frequency on the interpretation of the instantaneous rates.

In farm animals, measurements of respiratory exchanges and energy expenditure are generally conducted by indirect calorimetry using whole-body respiration chambers, hoods or face masks. Large chambers are most commonly used when measurements are conducted over several consecutive days. They are characterized by a much lower turnover rate of air than hoods or face masks (dependent on the volume of the chamber and its ventilation rate) resulting in steady-state conditions which can be attained after several hours. For example, a chamber of an inner gas volume ($\boldsymbol{V_G}$) of 4000 L in standard conditions of temperature and pressure can be ventilated by flow rates ranging from 50 to 250 L/min STP (depending on the respiratory ex-changes of the animal). The turnover rate of air expressed as $\boldsymbol{V/F} = \boldsymbol{\tau_G}$, would thus range from 15 to 73 min, implying that 95 % of the maximum response would be reached after 3 x $\boldsymbol{V/F}$ = 45 to 220 min respectively (Brown *et al.*, 1984). Such a characteristic is a handicap for measuring the energy expenditure associated with physiological phenomena of shorter duration such as eating, ruminating or standing in animals, and it then becomes necessary to account for this dilution phenomenon.

When measurements are carried out at frequent time intervals, the instantaneous rate of gas exchanges can be calculated, and several computation methods have already been published (McLean, 1972; Brown *et al.*, 1984; McLean and Tobin, 1987) that correct for the above mentioned dilution phenomenon. These methods include a differential term (accumulation term) which is based on the differences in gas concentrations in the chamber between the beginning and the end of the measurement intervals, as well as on the inner gas volume of the chamber.

The major drawback of these methods is a non-negligible variability which is introduced in the results of gas exchange rates (McLean, 1972 ; Brown *et al.,* 1984 ; McLean and Tobin, 1987). The latter actually originates from an amplification of measurement noises in both the inlet-outlet gas concentration differences and the chamber ventilation rate (McLean and Watts, 1976).

Physical measures can be taken to improve the precision of measurements in particular that of gas concentration differences which is most critical (McLean and Watts, 1976 ; Sun *et al.,* 1994). Additionally, the data acquisition system can be improved by largely increasing measurement frequency ; this must be coupled to a mathematical treatment of the data which deals with averaged values to smooth the amplified "measurement noises" (Sun *et al*., 1994). When measurements are highly frequent (of the order of a second or millisecond), average values of energy expenditure over prolonged periods do not seem to be greatly influenced by the nature of the differential term which is included in the calculation (Brown *et al*., 1984 ; McLean and Tobin, 1987).

However, improvements in the data acquisition and treatment systems are not always financially feasible. Such is the case with given experimental set-ups and with data acquisition systems which allow only discrete measurements of fixed frequencies of once per minute or lower frequencies (*e.g.* every 2, 3, …10 min) or when only one analytical plus acquisition system is connected to a series of respiration chambers allowing only alternative and infrequent measurements for each chamber. In those situations, it is still possible to improve the accuracy of fast-response energy expenditure results by selecting the best mode of calculation of the differential term which corrects for the dilution phenomenon (Brown *et al.,* 1984).

In case where data acquisition can only be made at time intervals in the order of minute, different mathematical treatments are possible concerning the differential term (accumulation term) of the balance equation. The consequence on the calculated instantaneous rates (oxygen uptake rate and CO_2 release rate) is not negligible leading to consider that evaluation of transient changes in metabolic activities (which is always the case for human being) is simple to assess.

Therefore, the consequences in terms of control strategy of the habitability of a closed chamber is not a simple task and cannot be treated neither with a zero-order control strategy, nor without a specific data treatment procedure (Ortigues *et al.*, 1997) accounting for all balance equations as presented in previous sections.

8 APPLICATION TO GREENHOUSES DESIGN

8.1 General and present approach of greenhouse design and modelling

Many attempts have been made to calculate the microclimate, especially the air exchange or ventilation for commercial greenhouses. Usually ventilation is used for replacing warm, humid, low-CO_2 greenhouse air with cooler, drier external fresh air. But the plant organs status, *i.e.* their temperatures, reactions and water stress, form the actual focus of the control system. Insufficient ventilation can lead to too high temperature, too low vapour pressure deficit or severe CO_2 depletion, all of which may be harmful to the plants. On the other hand, excessive ventilation may waste energy by necessitating additional heating (or artificial cooling in hot weather). Low humidity in the greenhouse, which may result from excessive ventilation, can cause high transpiration which creates water stress in plants (Fuchs *et al.*, 1995).

Basically, ventilation is generally adjusted to enable sufficient CO_2 input. But since plant transpiration plays a dominant role in the control of foliage and air temperatures in the greenhouse, the water status and transpiration of the plants must also be taken into consideration in programming the air (or gas) flow rates.

At present time, it is very difficult to predict the influence of the air flow and the air exchange rates created by either natural or forced ventilation in the commonly used greenhouses, because understanding of the physical processes that drive are not sufficiently advanced (Critten *et al.*, 2002 ; Bailey, 2000). There is little information on the coupling between the heat and mass exchanges between the plants and the ventilating air and their interactions with the physiological reactions of the plants makes the situation even more complicated (Critten *et al.*, 2002).

Sophisticated techniques have recently been developed for the visualization and quantification of air flows and transpiration in greenhouses (Boulard *et al.*, 1999 ; Montero *et al.*, 2000). They include the use of thin thermocouples on static grids (Lamrani *et al.*, 2001), sonic anemometers (Boulard *et* Wang, 2000). Recent advanced models for characterization and study of the fluxes have included elaborate computer software tools, such as computational fluid dynamics (CFD) programs (Bartzanas *et al.*, 2002 ; Haxaire *et al.*, 2000 ; Boulard *et al.*, 1999 ; Boulard et Wang, 2002 ; Mistriotis *et al.*, 1997). However, the main disadvantages of these models are their complexity and the fact that they do not necessarily take into account the retroactions between the plants and the air flow characteristics.

A simpler model that has been developed for use in a control system (Dayan *et al.*, 2004) is capable of describing the ventilation of a greenhouse system with adequate accuracy as required by the control. This model uses simple energy and mass balances. The model is based on the division of the greenhouse space into several horizontal compartments (or layers which are supposed to be perfectly mixed) exchanging matter and energy. The energy and vapor balances for the segments describe the micro-climate within these segments. The equations also account for the heat exchange between atmosphere and the canopy, which absorbs radiation and transpires.

This basic model is schemed in Figure 10.

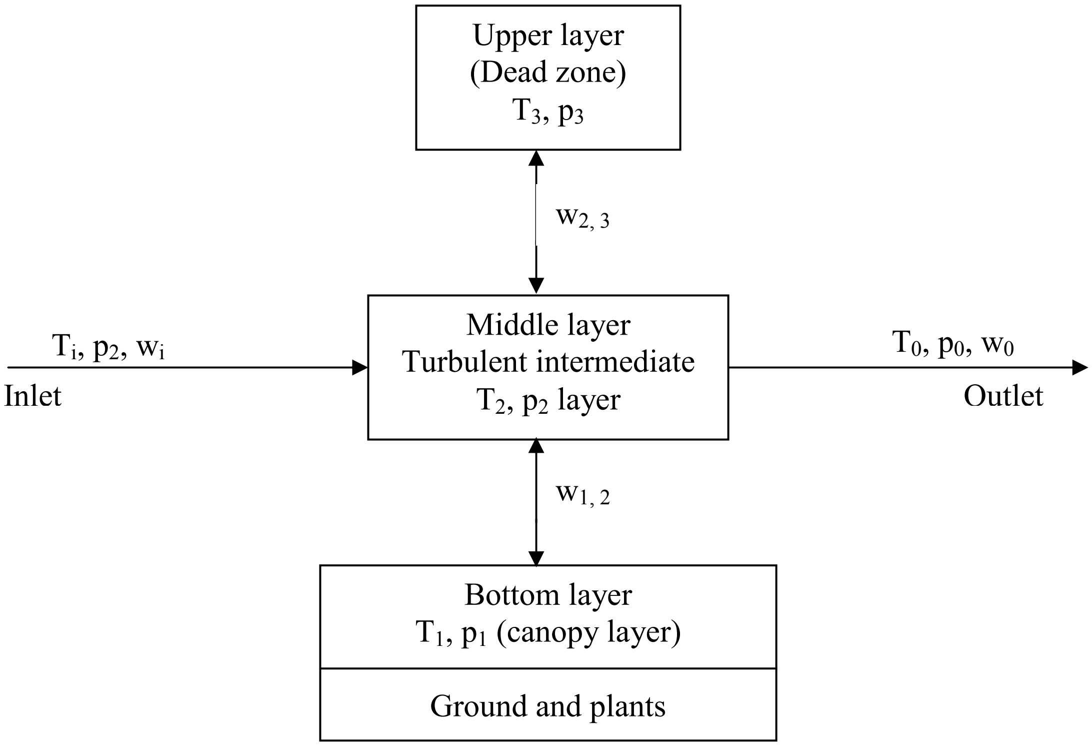


Figure 10 : Scheme of the reduced model of a gas phase in a greenhouse.

The ventilation model comprises three vertically stacked segments or layers, each assumed to be completely mixed. This partitioning is justified by the observed vertical temperature profile within the greenhouse.

- The bottom region consists of the layer of air that covers the entire canopy of the plants.
- The middle region consists of the layer of air above the plants and extending almost to the level of the roof of the greenhouse.
- The upper region accounts for the pocket layers which are the stagnant zones of air trapped in the dead zones.

In its simplest form, the basic model comprises 10 equations : three for dry air mass balance, three for water vapor (humidity), three for energy balances (one enthalpic balance for each segment) and an additional energy balance equation for the plants.

For the three segments, the balances are given in terms of ordinary differential equations. However, although airflows are assumed to be time varying, there is no air accumulation in any of the segments in such a way that total mass balance can be written with a pseudo-steady state assumption. In fact, it is sufficient to write that pseudo-steady state applies for total pressure, leaving the model the possibility to represent water accumulation that can be positive for an increase of vapor water content or negative for a decrease of vapor water content. In other words, this assumption consists in supposing that total pressure fluctuations, if they exist, are sufficiently rapid not to affect the total matter balance.

However, it must be considered that this assumption is not correct for estimating momentum transfer influence, which corresponds to the assessment of the influence of shear stresses on the development of the canopy by example. This last effect, which intuitively seems to be important for higher plants development, can only be accounted by estimation of air velocity profiles inside the greenhouse and air velocity fluctuations which are directly linked to shear stresses profiles. The only mean to assess this kind of information is, from a theoretical point of view, to use CFD simulation to be validated, from an experimental point of view, by both local rapid pressure measurements and local velocity profiles by anemometry (Bartzanas *et al.*, 2002 ; Haxaire *et al.*, 2000 ; Boulard *et al.*, 1999).

The other main assumption of the model is to consider that for a sufficiently short period (typically one day), the total mass of plants and the shape of the canopy do not change. This leaves the model to be recalibrated periodically for accounting for the increase of plant mass (or for the decrease of plants mass resulting from harvesting).

The resulting model is therefore valid on a time scale that is not too short (*ca* greater than one minute) and not long (*ca* less than one day) for representing the gas and energy exchanges between the canopy and the greenhouse atmosphere. The main interest of such a model is to leave unknowns that only concern the plant canopy balances, *i.e.* radiative balance with radiation uptake rate by the plants, mass balance with transpiration balance (water balance), O_2 and CO_2 balances. The experiments result in taking sufficient information in terms of temperature profiles, partial pressures profiles (gas concentrations) and flow rates in order these unknowns to be determined or identified by comparing simulation with experiments.

This kind of proceeding has a fundamental importance for linking plant physiology parameters (kinetic and conversion yields) to process parameters that are ventilation flow, gas partial pressures, total pressure. The shear stresses influence cannot be assessed by this kind of model knowing that CFD simulation and experimental validation remains necessary.

8.2 Objective of greenhouse modelling

The general objective of greenhouses modelling is not different from the objectives of modelling for the other compartments : *i)* to have an one-line representation of what happens in the subsystem, what will happen and what can be the trajectory of the subsystem in future ; *ii)* to use the previous representation for defining a suitable command law of the subsystem in order to fulfil and optimise an objective function which depends on the entire system objective.

Transposed to the greenhouse subsystem, the common attitude leads to consider that the previous two principles do not seem to be completely applicable. Roughly speaking, it is often considered that higher plants growth cannot be commanded, the only thing to be done being to provide the culture the suitable environment (acting on liquid and gas feeds) for having a maximum higher plants growth.

In fact, such an attitude reflects an important lack of knowledge of the rate limiting processes driving higher plants growth and metabolism.

As always, these processes must be spited into two categories : *i)* physiological organisation (and metabolism) considering the higher plant as a sum of several interacting parts (roots, leaves, foliage, stem...etc.) and knowing that the results must be integrated and averaged over a series of individual plants ; *ii)* physical rate limiting processes including radiation transfer, mass transfer (water, O_2 and CO_2 in the gas phase and other dissolved compounds in the liquid phase) and heat transfer, knowing that momentum transfer also has an influence on the morphology of the above ground part of the plant. As always also, the general objective of modelling is to give a representation, as thorough as possible, of the links existing between these various influences.

Coming back to the representation of the aerial part of plants which is in contact and interaction with the gas phase of the greenhouse, the model must correctly account for mass and heat exchanges between gas and above ground part of the plant, radiation transfer and temperature and composition gradients. The objective of the gas phase model is to correctly account for physically driven processes in order to be interfaced with physiology description.

In practice, this leads to consider :

- The radiative transfer between a light source and a canopy, knowing that already good estimations of light energy transfer at a canopy level are available but that this kind of global estimation are hardly transferred to one single plant.

- The mass tansfer kinetics between the leaves and the gas phase, including water, O_2 and CO_2 : this kind of calculation is easily achievable with a rough model using a mass transfer coefficient; however this transfer coefficient strongly depends on the velocity profile, the shape of the solid surfaces (plant leaves), the type of transfer (evaporation, condensation, or not) in such a way that the mass transfer coefficient must be considered as an empirical coefficient to be estimated and never extrapolated. The consequence is that, if no more sophisticated model is used, the predictability of the model is null, forbidding (or at least strongly reducing) any command law in order to reach an objective function. Therefore a deeper understanding of solid - gas transfer is required, knowing that the influence of gas exchanges on physiology is known to be important.

- The momentum transfer model, with three sub-objectives : *i)* to calculate the shear stresses applied to aerial parts of the plants ; *ii)* to use this description to assess a gas flow model inside the chamber between the different layers ; *iii)* to use the velocity profile at the vicinity of foliage walls for deriving predictive description of mass transfer between the leaves and the gas phase (see above).

Therefore, gas phase representation models must satisfy (progressively) the following conditions (from the simplest models to the most sophisticated models) :

- Assessment of gas exchange rates : production and consumption of O_2, CO_2 and transpiration rates and if possible on-line measurements with a (on-line) stoichiometric data reconciliation at the global greenhouse level.

- Assessment of radiation transfer to the canopy : global link between light energy input with photosynthetic activity.

- Assessment of concentrations (or partial pressures) and temperature heterogeneity inside the greenhouse; division of the gas phase greenhouse in several layers (see above) : first estimation of an empirical water transfer model.

- Assessment of radiative energy profile inside the canopy.

- Assessment of local mass and heat transfer at foliage levels to derive a predictive model for O_2, CO_2 and water exchanges between plants and gas phase.

- Assessment of gas velocity profile inside the greenhouse in order to derive a predictive modelling of flow exchanges between the different parts of the greenhouse : onset of a suitable compartmentalisation of the greenhouse space ; approach of the non terrestrial gravity on the intensity of the convective transfer.

- Modelling of gas boundary layers around the aerial parts of plants from the velocity profiles in order to derive a predictive influence of local and global gas partial pressures and therefore the influence of the total pressure ; approach of the influence of non terrestrial gravity of the boundary layers behaviors and therefore on the influence on mass transfer.

- Modelling of the influence of the mechanical constraints applied the aerial parts of plants.

Of course at all levels of modelling, the gas phase models must be connected to physiology models, at least at the leaves level, accounting for a progressively fine understanding of the interactions between vegetal cells and the gas phase.

Also, these modelling efforts must be compatible with experimental validations at all levels, from a global mass balances (respiration rates measurements) to local partial pressure measurements (MS measurements by example), total hydrostatic pressure measurements including pressure fluctuations and velocity profiles by anemometry.

Finally the global objective of gas phase modelling for a greenhouse is to derive a predictive representation of plant behavior accounting for :

i) light energy effects, including canopy density;
ii) gas partial pressure effects, including CO_2, O_2 and humidity;
iii) temperature gradient effects;
iv) mechanical constraints effects;
v) non terrestrial gravity effects.

8.3 Classification of the gas phase greenhouse models

The gradual objectives of the gas phase modelling and representation as previously defined correspond to a progressively increased complexity of the modelling. However, a model complexity cannot be increased in a so-called continuous way. It has rather to be considered as a “lego” type exercise, knowing that the degree of required complexity directly influences the general structure of the model. Contrariwise, knowledge models of various degree of depth must be kept in relation together in order to be compatible and gradually increase the predictability *i.e.* the decrease of the number of unknown empirical coefficients.

Therefore, the different models for the gas phase representation of a greenhouse can be classified in several classes. We discriminate four classes.

The first kind of model for the gas phase of a greenhouse is the one compartment model which consists in considering the gas phase as homogeneous with respect to all variables : temperature and gas partial pressures. This model must at least consider the stoichiometric constraints and the global influence of light energy input. It has to be parameterized (non predictive influence) with different variables such as total pressure, temperature, total gas flow rates...etc. From a technical point of view, input and output gas balances must be assessed (O_2, CO_2 and water balances).

The second class is the three layers model previously sketched. This model must account for gas phase heterogeneity, namely temperature gradients and gas partial pressures gradients, trying to improve a gas phase residence time distribution versus hydrodynamic agitation (fans, gas flow and natural convection). The main objective is to suitably link water exchanges (and CO_2 exchanges) versus agitation conditions (mechanical energy input) inside the greenhouse. From a technical point of view, it is necessary to have experimental evaluation of temperature (IR remote sensors) and gas partial pressures profiles (sampling tube connected with MS analysis) inside the greenhouse.

The third class is derived from the previous one by refining the description at the boundary layer level in order to correctly account for all exchanges at the foliage level. From a technical point of view, image data acquisition techniques should be useful for validation of such information.

The fourth class must use velocity profiles to derive the exchanges between the several layers of the previous model and at the boundary layer level. From a technical point of view, the experimental validation of such a description must be done with velocity profiles measurements (anemometry) and hydrostatic pressure measurements.

The general classification of the possible models of the gas phase of a greenhouse are summarized in Table 5.

Type of model	Assumption	Experimental validation	Objective
One compartment model	- Perfectly mixed gas phase - Homogeneous T and partial pressure in gas phase and equal to output stream values - Transfer of light energy to the surface of the canopy	- Measurement of input and output volumetric flow rates -Measurement of input and output gas partial pressures : p_{O2}, p_{CO2}, humidity - Measurement of total pressure in the greenhouse and average T - Measurement of light energy production	- Global mass balance analysis - Stoichiometric reconciliation - Total energy balance
3-layers model : ■ Canopy layer ■ Turbulent intermediate layer ■ Dead zone layer	- Each layer perfectly mixed - Exchange gas flow between the 3 layers	- Local T measurement - Local partial pressure measurement by gas sampling and MS analysis for p_{O2}, p_{CO2}, p_{H2O} and other gas	- Characterisation of local (at foliage level) influence of T and partial pressures - Characterisation of gas RTD versus agitation devices (fans) - Characterisation of the in-depth penetration of light energy inside the canopy
Boundary layer model	- Stagnant gas boundary layer at leaves surface - Exchange of heat and mass with bulk canopy layer (see previous model) by diffusive transfer	- Image analysis of aerial part of plants	- Predictive transfer model mass and heat exchanges between leaves and surroundings
Complete hydrodynamic model	- Classical assumptions for gas turbulence models - Stagnant boundary layers and solid surfaces - Caricatural representation of solid shapes (plants, lamps, fans) into the green house	- Gas velocity profiles - Total hydrostatic pressures	- To link the previous models with CFD simulation - To take into account a predictive approach of convective terms - To attempt to obtain a predictive approach of non-terrestrial gravity

Table 5 : Classification of gas phase model for a greenhouse ; the models must be kept in connection knowing that a more elaborated model must at least keep the results of the previous ones.

9 CONCLUDING REMARKS AND RECOMMENDATIONS

All the mass and energy balances have been presented in the previous sections and the applications to modelling and control of metabolic chambers, either closed habitats for human activities or higher plant chambers, have been reviewed.

Major issues emerge from this analysis. This mainly concerns :

- *(i)* the choice of instrumentation that must be selected for analysing gas concentrations,
- *(ii)* the mathematical treatment that must be done with experimental data, either for assessing metabolic variables or for applying a control strategy,
- *(iii)* the understanding, *i.e.* the modelling, of rate limiting processus when those are coming from physical transfer.

The following points are particularly important :

- Assessment of instantaneous metabolic rates R_{ni} is possible from gas phase analysis. The values can be used for implementing a control strategy. The values are reliable for gaining a global on-line information and understanding of metabolic activity (either concerning higher plants or human activity) but the implementation of data treatment and analysers must be very carefully done.

- The respiration rate can only be assessed if the gas mole fractions are measured with a high accuracy, *i.e.* at 0.1 % = ± 500 ppm for oxygen and 0.005 % = ± 25 ppm for CO_2. They must be measured both at inlet and outlet gas streams. Water vapor content in gas stream at measurement points must carefully studied. Similar conclusions for any other released or consumed gas component can be obtained.

- For a metabolic chamber a further degree of complexity must be considered : the gas accumulation term cannot generally be neglected. This situation comes from the fact that the gas residence time is generally much higher than in classical bioreactors (several minutes for classical metabolic chambers). This entails that specific data treatments must be performed accounting for transient terms in the mass balance equations and that the measurements frequency may impact the values of the rates that are calculated.

- The cross flow of liquid (or the accumulation of biomass into chamber) must generally be considered, accounting for gas solubility in the mass balances. For permanent gas, the solubilities being low and when liquid cross flow is low, the correction is not very significant. However, for other organic gas, and even for CO_2, this must be considered keeping in mind that solubility depends on temperature and in most cases of pH.

- Water balance remains a very difficult point to assess. The difficulties come from several problems. *(i)* The inlet and outlet water gas stream content is difficult to assess; it strongly depends on temperature and generally it is mandatory to remove water content before any other gas analysing device. *(ii)* Of course, liquid balance cannot be ignored, which means that liquid water accumulation must be considered; this in turns leads to take into account the condensation points. *(iii)* Accumulation of water into biomass (*c.a.* 70 % of wet mass of plants by example) must be considered.

- A first mathematical approach consists in considering a perfectly mixed and homogeneous gas phase. This is generally satisfactory for the estimation of global metabolic rate $\boldsymbol{R_{ni}}$ from gas mole fractions and flow rate data. This is completely true if gas accumulation terms can be neglected; however, the assumption becomes more and more inaccurate if the heterogeneity of the gas phase increases and if the accumulation of gas compounds in some regions of the chamber is very different from the average value.

- In terms of modelling and understanding, the assumption of homogeneous gas phase is far to be correct if knowledge models of physical rate limited process must be considered. A first departure model, basically considering a gaseous or a liquid boundary layer, has been put on the table. This model includes a volumetric transfer coefficient $\boldsymbol{K_L a}$. Although it is a rough model, it may be a clue for studying and understanding the metabolic effect of hypobaric growth of higher plants. It must also be used as a departure model for studying the water exchanges between higher plants and their surroundings.

- In terms of modelling and control of habitability, the assumption of homogeneous gas phase is certainly not a valuable assumption. The gas concentration gradients may be important. A very simple estimation leads to consider that the smallest the gas concentration of one component, the highest the gradient (in relative units) from point to point. This true (and well known) for CO_2 but also for any trace compound and for water vapour, leading in this last case to “suprising” condensation or evaporation. This must necessarily be considered in order to suitably understand and control the local gas concentrations (including water) in the vicinity of living organisms. This is also known as a controlling process (in food industry by example) the biofilm growth on solid surfaces.

- A promising way to develop non-homogeneous description of gas compartment volume of a metabolic chamber might be to use CFD for defining several (pseudo-homogenous) zones, keeping in mind that the gas / solid interfaces must be specifically treated. This is also the only way for computing the mechanical shear stress which is known to have an effect on higher plants growth. Such models, which include gravitational effects, would be the good way to give a first insight (to provide a prediction ?) of the macroscopic effect of non terrestrial gravity on plant growth.

Notation

Φ :	Flow rate	$kg.s^{-1}$; $m^3.s^{-1}$; $mole.s^{-1}$; $J.s^{-1}$ …
μ :	Specific internal energy (per unit man)	$J.kg^{-1}$
h :	Specific enthalpy (per unit man)	$J.kg^{-1}$
s :	Specific entropy (per unit man)	$J.kg^{-1}K^{-1}$
C_p :	Heat capacity (per unit man)	$J.kg^{-1}K^{-1}$
p :	Absolute pressure	Pa : 1 atm = 101.3 kPa
T :	Temperature	K : 0°C = 273.15 K
y_i :	Gas phase molar fraction of component i	
p_i :	Partial pressure of component I	Pa
R :	Ideal gas constant	$R = 8.3143\ J.mole^{-1}.K^{-1}$
m :	Total mass in the system	kg
n :	Total number of moles in the system	mole
R_n :	Production ($R_n > 0$) or consumption ($R_n < 0$) rate in system	$mole.s^{-1}$
R_{ni} :	Production or consumption rate of component i in system	$mole.s^{-1}$
V :	Total gas volume of the system	m^3
Φ_L :	Liquid flow rate (volumic)	$m^3.s^{-1}$
C_i :	Concentration of component i in liquid flow	$mole.m^{-3}$
r_{ni} :	Volumetric production ($r_{ni} > 0$) or consumption ($r_{ni} < 0$) rate in system	$mole.\ m^{-3}.s^{-1}$
M :	Molecular weight	$g.mole^{-1}$
w_i :	Gas phase mass fraction	

Subscripts

n : molar units
m : mass units
v : volumic units
i : component i
L : Liquid flow
h : enthalpy
s : entropy
g : Gibbs energy

Superscripts

(1) : inlet flow
(2) : outlet flow

References

BAILEY B.-J. (2000) Constraints. *Acta Hort.* **534:** 21-30.

BARTZANAS T., BOULARD T., KITTAS, C. (2002). Numerical simulation of airflow and temperature distribution in tunnel greenhouse equipped with insect-proof screen in the openings. *Comput. Electron. Agric.* **34:** 207-221.

BOULARD T., HAXAIRE R, LAMRANI M., ROY J.-C., JAFFRIN A. (1999). Characterization and modeling of the air fluxes induced by natural ventilation in greenhouse. *J. Agric. Eng. Res.* **74:** 135-144.

BOULARD T., WANG S. (2000) Greenhouse crop transpiration simulation from external climate conditions. *Agric. For. Meteorol.* **100:** 25-34.

BOULARD T., WANG S. (2002). Experimental and numerical studies on the heterogeneity of crop transpiration in a plastic tunnel. *Comput. Electron. Agric.* **34:** 173-190.

BOULARD T., WANG S., HAXAIRE R. (2000). Mean and turbulent air flows and microclimatic patterns in an empty greenhouse tunnel. *Agric. For. Meteorol.* **100:** 169-181.

BROWN D., COLE T.-J., DAUNCEY M.-J., MARRS R.-W., MURGATROYD, P.-R. (1984). Analysis of gaseous exchange in open-circuit indirect calorimetry. *Med. & Biol. Eng. & Comp.* **22:** 333-338.

CRITTEN D.-L., BAILEY B.-J. (2002). A review of greenhouse engineering developments during the 1990s. *Agric. For. Meteorol.* **112:** 1-22.

DAYAN J., DAYAN E., STRASSBERG Y., PRESNOV E. (2004). Simulation and control of ventilation rates in greenhouses. *Math. Comput. Simul.* **65**: 3-17.

FUCHS M., SEGAL I., DAYAN E., JORDAN K. (1995). Improving Greenhouse Microclimate Control with the Help of Plant Temperature Measurements, IS-1816-90r.

HAXAIRE R., BOULARD T., MERMIER M. (2000). Greenhouse natural ventilation by wind forces. *Acta Hort.* **534:** 31-40.

LAMRANI M., BOULARD T., ROY J.-C. (2001). Air flows and temperature patterns induced in a confined greenhouse. *J. Agric. Eng. Res.* **28:** 75–88.

MCLEAN J.-A. (1972). Analysis of the efficiency of greenhouse ventilation using computational fluid dynamics. *Agric. For. Meteorol.* **85:** 217-228.

MCLEAN J.-A., TOBIN G. (1987). *Animal and Human Calorimetry.* Cambridge: Cambridge University Press.

MCLEAN J.-A., WATTS P.-R. (1976). Analytical refinements in animal calorimetry. *J. Appl. Physiol.* **40:** 827-831.

MISTRIOTIS A., BOT G.-P.-A., SEARASEIA-MUGNOZZA G., PICUNO P. (1997) Analysis of the efficiency of greenhouse ventilation using computational fluid dynamics. *Agric. For. Meteorol.* **85:** 217-228.

MONTERO J.-I., ANTON, A. (2000). Buoyancy driven ventilation in tropical greenhouses. *Acta Hort.* **534:** 41-48.

ORTIGUES I., DUSSAP C.-G., ANGLARET Y. (1997). Comparison of various methods for calculating the instantaneous respiratory gaseous exchanges from discrete measurements in respiration chambers. *J. Theor. Biol.* **185:** 489-501.

SUN M., REED G.-W, HILL J.-O. (1994). Modification of a whole room indirect calorimeter for measurement of rapid changes in energy expediture. *J. Appl. Physiol.* **76:** 2686-2691.