

***Method for the Statistical Treatment of Elemental and Energy
Balances with Application to Steady-State Continuous-Culture Growth
of *Saccharomyces cerevisiae* CBS 426 in the Respiratory Region***

INTRODUCTION

The application of elemental balances to the description of bioengineering systems has become an accepted tool, the theory of which has been developed in a number of important publications.¹⁻⁴ One of the problems of the application of elemental balances is the occurrence of errors, which are associated with the measurement of the flows to and from a system. These errors are of two types: bias, i.e., a systematic deviation between measured and actual flows, and random errors in the measurement. In most systems commonly studied, the number of measured flows is such that the elemental balances present an overdetermined set of equations. In this case the information present in the formulation of the elemental balances can be used to improve the estimate of the actual flows obtained by measurement. The reasoning presented in this paper is an extension of the method developed by Madron et al.⁵ and Madron.⁶ In this paper the general case is treated and it is shown how the enthalpy balance can be integrated into the formulation of one general set of conservation principles. An application to the aerobic growth of a yeast and the optimal estimation of the respiratory quotient (RQ) is shown.

THEORY

A convenient starting point for the description of an open system is the definition of the chemical state vector, C , of that system. If the system is considered to contain n chemical species, C is a column vector of dimensionality n . Within this vector the concentration of each chemical species in the system is identified by a number. It can be shown⁷ that an equation for the time evolution of the chemical state vector can, for a system of constant volume without concentration gradients, be written

$$\frac{dC}{dt} = \alpha \cdot r + \phi \quad (1)$$

where α is the stoichiometry matrix of the system an $n \times m$ matrix; r is the vector of reaction rates of the m transformation processes taking place in the culture, it is an $m \times 1$ column vector; ϕ (an $n \times 1$ column vector of the flows of the various chemical species to the system) is expressed as mol/m³ system volume.

For a culture of one or more microorganisms, it is safe to assume that chemical elements are conserved as far as the reaction pattern is concerned. An efficient matrix equation, summarizing all elemental balances, is obtained if the right- and left-hand sides of eq. (1) are multiplied by a so-called elemental composition matrix E . The matrix E is a $k \times n$ matrix in which the columns contain the number of each of the chemical elements present in one molecule of the chemical compound under

consideration:

$$\mathbf{E} = \begin{bmatrix} a_{11} & a_{1j} & a_{1n} \\ a_{i1} & a_{ij} & a_{in} \\ a_{k1} & a_{kj} & a_{kn} \end{bmatrix} \quad (2)$$

where a_{ij} is the number of atoms of chemical element i present in the j th chemical compound:

On multiplication of the left- and right-hand sides of eq. (1) by \mathbf{E} it follows:

$$\frac{d\mathbf{B}}{dt} = \mathbf{E} \cdot (\boldsymbol{\alpha} \cdot \mathbf{r}) + \mathbf{E} \cdot \boldsymbol{\phi} \quad (3)$$

where \mathbf{B} is a $k \times 1$ column vector that consists of the amounts of the various chemical elements present in the system (g atoms/m³). By virtue of the fact that the amounts of the various chemical elements are conserved quantities, their net rates of synthesis must equal zero, hence it follows from eq. (3):

$$\mathbf{E} \cdot (\boldsymbol{\alpha} \cdot \mathbf{r}) = 0 \quad (4)$$

For the present analysis it is desirable to state the conservation principle according to eq. (4) in terms of the vector of flows $\boldsymbol{\phi}$, as this vector can be readily studied experimentally. For a system in a steady state it follows from eq. (3):

$$\mathbf{E} \cdot \boldsymbol{\phi} = -\mathbf{E} \cdot (\boldsymbol{\alpha} \cdot \mathbf{r}) \quad (5)$$

Or, by combination of eqs. (4) and (5):

$$\mathbf{E} \cdot \boldsymbol{\phi} = 0 \quad (6)$$

This formulation of the concept of the elemental balance according to eq. (6) will be used throughout this paper. Equation (6) can be used to perform elemental balances on the measured flows of, for example, substrate, biomass, oxygen, and CO₂ for organisms growing in a steady state in a continuous culture. However, a situation frequently arises in which the number of flows measured is so large that the system of equations obtained by application of eq. (6) becomes overdetermined, i.e., more equations are obtained than there are unknown flows left. In this case, inconsistencies may arise between the various elemental balances and it becomes unclear which of the measured flows is subject to inaccuracies and to what extent. Two types of flows are distinguished in the treatment, the flows $\boldsymbol{\phi}'$ that have been measured, and the flows, $\boldsymbol{\eta}$, that have not been measured.

The conservation equation [eq. (6)] is thus rewritten

$$\mathbf{E}' \cdot \boldsymbol{\phi}' + \mathbf{E}'' \cdot \boldsymbol{\eta} = 0 \quad (7)$$

where \mathbf{E}' is the elemental composition matrix for the flows that have been measured and \mathbf{E}'' is the elemental composition matrix for the flows that were not measured.

The measured values of the flows are, in most cases, not equal to the real values of the flows, given by the vector $\boldsymbol{\phi}'$, and the conservation principle according to eq. (7) only holds for the real flows. We will assume that the measured values of the flows are given by a vector $\boldsymbol{\epsilon}$, a vector of the same dimensionality as the vector $\boldsymbol{\phi}'$.

The measured flows are assumed only to contain random error, and the variance-covariance matrix of the measurements, F , of the flows is assumed to be known.

A vector of errors of the measured flows with respect to the actual flows is given by:

$$e = \phi' - \epsilon \quad (8)$$

The whole problem is now reduced to finding the most probable values of the errors, e . As the variance-covariance matrix of the measured flows is known, a realistic objective function to be minimized seems to be:

$$O = e^T \cdot F^{-1} \cdot e \quad (9)$$

where F^{-1} is the inverse matrix of the variance-covariance matrix.

For the case in which the off-diagonal elements of the variance-covariance matrix are zero, i.e., if there is no covariance between the measured values of the flows, eq. (9) is easily identified as the sum of the squares of the deviations between actual and measured flows, weighted according to the reciprocals of the squared standard errors of the flows.

The problem becomes now to minimize O subject to the constraints posed by eq. (7). This may be solved in a straightforward manner as is shown in the Appendix. The following equations result:

$$e = -F \cdot (E')^T \cdot [E' \cdot F \cdot (E')^T]^{-1} \cdot (E' \cdot \epsilon + E'' \cdot \eta) \quad (10)$$

$$\eta = -\{(E'')^T \cdot [E' \cdot F \cdot (E')^T]^{-1} \cdot E''\}^{-1} \cdot (E'')^T \cdot [E' \cdot F \cdot (E')^T]^{-1} \cdot E' \cdot \epsilon \quad (11)$$

From eqs. (10) and (11) the most probable errors and the most probable values of the unknown flows are obtained. Equation (8) can then be used to estimate the most probable values of the real flows.

As will be shown in the next section, this procedure is very effective in the correction of measured flows by the elemental balance principle.

The treatment can further be generalized by the introduction of the enthalpy balance. Enthalpy is, as the amounts of the various elements are, a conserved quantity. In cases where the flow of heat to the system is measured, the enthalpy balance can be used to formulate an additional constraint in the sense of eq. (7).

If the heat flow is not measured and the system is overdetermined, the procedure can be applied to the estimation of the heat flow. The enthalpy balance can be easily introduced into the present treatment if the vector of flows is augmented by the heat flow to the system and one additional row and column are added to the matrix E :

$$E^* = \begin{bmatrix} a_{11} & \cdots & a_{1j} & \cdots & a_{1n} & 0 \\ a_{i1} & \cdots & a_{ij} & \cdots & a_{in} & 0 \\ a_{k1} & \cdots & a_{kj} & \cdots & a_{kn} & 0 \\ h_1 & \cdots & h_j & \cdots & h_n & 1 \end{bmatrix} \quad (12)$$

The column consists of n zeros and a 1, the row consists of the molar enthalpies of the components of the system and a 1. If the matrix E^* is used instead of the matrix E , the procedure developed above can be applied to cases when the enthalpy balance is also considered.

MATERIALS AND METHODS

The continuous fermentation and the analytical methods have been previously described.⁸

RESULTS AND DISCUSSION

For the application of elemental balances, it is necessary to know the atomic composition of the biomass. In balance studies an average elemental composition $\text{CH}_{1.83}\text{O}_{0.56}\text{N}_{0.17}$ was used.

The steady-state flows of oxygen, carbon dioxide, substrate, and biomass were measured several times for each dilution rate and the results were used to calculate average flows and the variance-covariance matrix of the flows to be used in the algorithm described in the theory. The consistency of the carbon balance was checked in order to detect a possible bias in the measured values of the flows. The difference between ingoing and outgoing flows of carbon averaged -2.5% ; considering the variance of the measurements, this can be shown not to be significantly different from zero.

The algorithm described in the Theory section was applied to the raw experimental data and the corrected flows thus obtained are recorded in Table I. (A copy of the computer program used can be obtained from the authors.) The average corrections applied to the flows were 0.006 for glucose, 0.01 for oxygen, and -0.037 for carbon dioxide, or 0.3, 1.2, and -3.7% . This again indicates a minor contribution of bias as the relative standard deviations of the measurements are 6, 11.7, and 11.1%, respectively.

An important feature of the method becomes clear when the RQ (respiratory quotient, i.e., the absolute value of the ratio between the flow of carbon dioxide and the flow of oxygen) calculated from the raw data is compared to the RQ from the corrected flows (Table II). As can be seen, the average of the corrected RQ's is different from that of the directly calculated RQ's and the standard deviation of the former is smaller than that of the latter. This is due to four high RQ values caused by anomalously high errors in the CO_2 flow (in three instances) or the O_2 flow (in one instance). It will be clear that in all cases the RQ calculated according to the corrected values will give a much better impression of the metabolism of the organism than the directly calculated value.

The algorithm developed here is particularly useful in the application of the RQ control in the bakers' yeast fermentation as proposed by, among others, Wang et al.⁹ This algorithm allows recognition of ethanol formation earlier and with greater reliability than when the measured flows are used directly. If the ethanol is included in the vector of unknown flows, the algorithm can be used to determine whether this flow is significantly different from zero, by performing an *F*-test on the objective function *O*.

In general, the algorithm is useful in the calculation of unknown flows of products or substrates and in the detection of equipment errors. In both cases, one or more of the flows will be corrected systematically.

APPENDIX

The minimization of the objective function *O* under the constraints posed by the elemental balance equations.

$$O = \mathbf{e}^T \cdot \mathbf{F}^{-1} \cdot \mathbf{e} \quad (13)$$

TABLE I
Dry Weight (DW) and Glucose Consumption Rate, Oxygen
Consumption Rate, and Carbon Dioxide Production Rate per Unit of
Biomass Produced of a Glucose-Limited Continuous Culture of *S.*
cerevisiae CBS 426

D (hr ⁻¹)	DW (g/liter)	ϕ_{glucose} (C mol/C mol biomass)	ϕ_{O_2} (mol/C mol biomass)	ϕ_{CO_2} (mol/C mol biomass)	
0.008	3.6	2.0	1.1	1.4	a
		2.18	1.09	1.18	b
0.008	3.8	2.1	3.8	1.4	a
		2.39	1.34	1.39	b
0.017	3.2	2.3	1.5	1.6	a
		2.50	1.43	1.49	b
0.033	4.0	1.9	1.04	1.1	a
		2.06	0.99	1.06	b
0.047	3.17	2.48	1.2	1.2	a
		2.42	1.19	1.30	b
0.052	4.2	1.8	0.82	0.86	a
		1.87	0.81	0.86	b
0.072	4.41	1.74	0.54	0.73	a
		1.69	0.63	0.69	b
0.076	4.0	1.93	0.67	0.73	a
		1.73	0.68	0.73	b
0.092	4.4	1.8	0.7	0.7	a
		1.73	0.67	0.73	b
0.092	3.8	1.9	0.7	0.8	a
		1.80	0.74	0.80	b
0.102	4.4	1.8	0.62	0.85	a
		1.70	0.65	0.71	b
0.112	3.6	2.1	0.78	0.92	a
		1.94	0.88	0.94	b
0.113	4.3	1.8	0.8	1.0	a
		1.88	0.83	0.89	b
0.118	4.5	1.71	0.57	0.67	a
		1.66	0.60	0.66	b

^a Raw experimental data.

^b Corrected values.

TABLE II
Comparison of RQ Values Calculated from Raw
and Corrected Data

Dilution rate	RQ	
	raw data	corrected data
0.008	1.27	1.082
0.008	2.71 ^a	1.037
0.017	1.066	1.041
0.033	1.057	1.070
0.047	1.000	1.092
0.052	1.048	1.061
0.072	1.35	1.095
0.076	1.090	1.073
0.092	1.000	1.089
0.092	1.142	1.081
0.102	1.370	1.092
0.112	1.179	1.068
0.113	1.25	1.072
0.118	1.175	1.100
Average	1.153 (0.118) ^b	1.075 (0.019) ^b

^a Considered to be an outlier, removed in the statistical treatment.

^b Value in parentheses is the standard deviation.

must be minimized under the constraints

$$\mathbf{E}' \cdot \boldsymbol{\phi}' + \mathbf{E}'' \cdot \boldsymbol{\eta} = 0 \quad (14)$$

with

$$\boldsymbol{\phi}' = \boldsymbol{\epsilon} + \mathbf{e} \quad (15)$$

A vector of Lagrange multipliers $\boldsymbol{\lambda}$, being a column vector of dimensionality k (k being the number of elements considered), is introduced.

The problem stated can now be formulated as:

Minimize O' with

$$O' = \mathbf{e}^T \cdot \mathbf{F}^{-1} \cdot \mathbf{e} - \boldsymbol{\lambda}^T \cdot (\mathbf{E}' \cdot \boldsymbol{\phi}' + \mathbf{E}'' \cdot \boldsymbol{\eta}) \quad (16)$$

This minimum is obtained if:

$$\frac{\partial O'}{\partial \mathbf{e}} = 0 \quad (17)$$

and

$$\frac{\partial O'}{\partial \boldsymbol{\eta}} = 0 \quad (18)$$

Thus:

$$2\mathbf{e}^T\mathbf{F}^{-1} = \boldsymbol{\lambda}^T \cdot (\mathbf{E}') \quad (19)$$

$$\boldsymbol{\lambda}^T \cdot \mathbf{E}'' = 0 \quad (20)$$

From eq. (19) it follows:

$$\mathbf{e} = \frac{1}{2}\mathbf{F} \cdot (\mathbf{E}')^T \cdot \boldsymbol{\lambda} \quad (21)$$

On substitution of eq. (21) into eq. (14) considering eq. (15)

$$\boldsymbol{\lambda} = -2[\mathbf{E}' \cdot \mathbf{F} \cdot (\mathbf{E}')^T]^{-1} \cdot (\mathbf{E}' \cdot \boldsymbol{\epsilon} + \mathbf{E}'' \cdot \boldsymbol{\eta}) \quad (22)$$

From eqs. (22) and (21):

$$\mathbf{e} = -\mathbf{F} \cdot (\mathbf{E}')^T \cdot [\mathbf{E}' \cdot \mathbf{F} \cdot (\mathbf{E}')^T]^{-1} \cdot (\mathbf{E}' \cdot \boldsymbol{\epsilon} + \mathbf{E}'' \cdot \boldsymbol{\eta}) \quad (23)$$

and, with eqs. (20) and (22)

$$\boldsymbol{\eta} = -\{(\mathbf{E}'')^T \cdot [\mathbf{E}' \cdot \mathbf{F} \cdot (\mathbf{E}')^T]^{-1} \cdot \mathbf{E}''\}^{-1} \cdot (\mathbf{E}'')^T \cdot [\mathbf{E}' \cdot \mathbf{F} \cdot (\mathbf{E}')^T]^{-1} \cdot \mathbf{E}' \cdot \boldsymbol{\epsilon} \quad (24)$$

Nomenclature

a_{ij}	number of atoms of chemical element i present in the j th chemical compound
\mathbf{B}	vector that consists of the amounts of the various chemical elements present in the system (g atoms/m ³)
\mathbf{C}	chemical state vector
\mathbf{e}	vector of the errors
\mathbf{E}	elemental matrix
\mathbf{E}'	elemental composition matrix for the flows that have been measured
\mathbf{E}''	elemental composition matrix for the flows that have not been measured
\mathbf{E}^*	elemental composition matrix extended by a row of molar enthalpies
\mathbf{F}	variance-covariance matrix
h_i	molar enthalpy of the i th component of the system
O	objective function
\mathbf{r}	vector of the reaction rates
RQ	respiratory quotient
$\boldsymbol{\alpha}$	stoichiometry matrix of the system
$\boldsymbol{\epsilon}$	vector of the measured values of the flows
$\boldsymbol{\eta}$	vector of the flows that have not been measured
$\boldsymbol{\phi}$	vector of the flows to the system
$\boldsymbol{\phi}'$	vector of the flows that have been measured

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