Material–Balance Calculations of Fermentation Processes

INTRODUCTION

The material balance represents a basis for calculating the consumption or formation of species that could not be determined during a fermentation process. Most of the hitherto published papers are devoted to calculations of oxygen consumption[1–5] and of respiratory quotient[4,5] in the process of single-cell protein manufacture. More recent studies by Cooney et al.[6] and Wang et al.[7] deal with the application of balance calculations for computerized process control.

The present paper aims at formulating the limitations as well as some further possibilities of the use of balance calculations of fermentation processes.

FORMULATION OF THE PROBLEM

Let a fermentation process be considered, in which I species composed of J elements ($E_1, \ldots, E_j, \ldots, E_J$) take part either as starting raw materials or products. The elementary composition of all such species can be advantageously expressed by the atom matrix $\mathbf{E} = \{e_{ji}\}$, where e_{ji} is the number of atoms of an element E_j in a molecule of the ith species. In this case a species is described by a so-called empirical formula, which need not be in relation to the structure of that species. Readers who are not familiar with matrix algebra applied to material balances may refer to recent papers on this subject.[8,12]

The fundamental relations for material–balance calculations are the elemental conservation equations

$$\sum_{i=1}^{I} e_{ji} n_i = 0; \qquad j = 1, \ldots, J \qquad \text{or } \mathbf{E} \cdot \mathbf{n} = \mathbf{0} \tag{1}$$

where n_i is the change in the number of moles of the ith species due to the fermentation process (with positive or negative sign in the case of product or raw material, respectively).

Let us assume that based on the independently specified values $n_1, \ldots, n_S$ (vector $\mathbf{n}_s$), one needs to calculate the remaining values $n_{S+1}, \ldots, n_I$ (vector $\mathbf{n}_c$); hereafter, such procedure will be referred to as a material–balance calculation.

Further, let us consider factors limiting the choice of species, for which $\mathbf{n}_c$ can be calculated on the basis of eq. (1).

THEORETICAL

Let us assume, with a view to simplicity, that the maximum number of linearly independent rows (i.e., rank) of a matrix $\mathbf{E}$ is equal to the number of elements J. Concerning the fermentation processes, this assumption is usually satisfied.

Equations (1) can be rearranged by separating the independently determined values $\mathbf{n}_s$

$$\sum_{i=S+1}^{I} e_{ji} n_i = -\sum_{i=1}^{S} e_{ji} n_i \quad \text{or} \quad \mathbf{E}_c \cdot \mathbf{n}_c = -\mathbf{E}_s \cdot \mathbf{n}_s \tag{2}$$

where the matrix $\mathbf{E}_s$ is obtained from the first S columns of the matrix $\mathbf{E}$, whereas $\mathbf{E}_c$ consists of the remaining columns.

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Now, depending on the amount of information contained in the values $\mathbf{n}_s$ and on the solvability of the system of eqs. (2) with respect to $\mathbf{n}_c$, four cases may arise according to the number and choice of the specified species:

1) Information contained in $\mathbf{n}_s$ is sufficient for the determination of $\mathbf{n}_c$.

2) Information contained in $\mathbf{n}_s$ is not sufficient for the determination of $\mathbf{n}_c$.

Simultaneously, one of the two following cases can occur:

a) Equations (2) are solvable for $\mathbf{n}_c$.

b) Equations (2) are not solvable for $\mathbf{n}_c$; in this case we state that the choice of specified values was not consistent.

For practical purposes only case 1 is significant. Let us consider the consistent case 1(a) first.

The material-balance eqs. (1) represent $r(\mathbf{E}) = J$ independent relations for the calculation of the values $\mathbf{n}_c$. It is, therefore, possible to choose independently $I - J$ components of a vector $\mathbf{n}$, whereas the remaining J components of the vector $\mathbf{n}$ can be calculated from eqs. (2). The selection of the values to be calculated must satisfy the following condition.[8]

$$r(\mathbf{E}_c) = r(\mathbf{E}) \tag{3}$$

Since the number of calculated species C is equal to J, $\mathbf{E}_c$ is a regular square matrix and the system of eqs. (2) then can be solved directly:

$$\mathbf{n}_c = -\mathbf{E}_c^{-1} \cdot \mathbf{E}_s \cdot \mathbf{n}_s = \mathbf{A} \cdot \mathbf{n}_s \tag{4}$$

Today, when subroutines for matrix operations are part of the software of even the smallest computers, eq. (4) offers a simple and universal solution to balance calculations.

If the values $\mathbf{n}_s$ are obtained experimentally, they are subjected to errors. Let us assume that the measured values $\mathbf{n}_s$ are subjected only to random errors with zero mean value and with normal distribution. When these assumptions are satisfied, the accuracy of the calculated values is expressed by the following simple relation:

$$\mathbf{W}_c = \mathbf{A} \cdot \mathbf{W}_s \cdot \mathbf{A}^T \tag{5}$$

where $\mathbf{W}_c$ is the covariance matrix of errors of calculated values $\mathbf{n}_c$ and $\mathbf{W}_s$ is the covariance matrix of experimental errors of measured values.

The inconsistent case 1(b) may occur if it holds that

$$r(\mathbf{E}_c) < r(\mathbf{E}) \tag{6}$$

and

$$C = r(\mathbf{E}_c) \tag{7}$$

when too many species have been specified. If this is caused by an incorrect formulation of the problem (e.g., in the case of a design calculation), it is necessary to reduce the number of specified species in order to obtain a consistent case. By not using some of the values of $\mathbf{n}_s$ obtained by measuring, however, some of the information is lost. In such a case it is more advantageous to carry out a statistical smoothing of the measured data so that the system (2) becomes solvable.[9]

EXAMPLE

Let us consider the process of the manufacture of single-cell protein from ethanol.[5,11] Essentially eight species are taking part in the process of fermentation: biomass (general formula: $C_{3.83}H_{7.00}O_{1.94}N_{0.64}Ah_{7.00}$, Ah being a fictive element representing the inorganic component—ash—of the biomass), ethanol, by-produced

acetic acid, oxygen, carbon dioxide, ammonia, water, and mineral nutrients (also designated as Ah).

These eight species are composed from five elements: C, H, O, N, and Ah. The corresponding atom matrix **E** is shown in Table I. Rank of the matrix **E** is equal to the number of elements, i.e., 5. Based on the material balance it is possible to calculate n_i for five suitable selected species. Now, let us consider some possibilities of the choice of specified species.

Biomass, NH_3, Ah: C = 5, $r(\mathbf{E}_c) = 3$. Too little information, the choice of specified species is inconsistent ($\mathbf{n}_s$ components are fixed by the balance of N and Ah). The other n_i values cannot be obtained by calculation (case 2(b)).

Biomass, ethanol: C = 6, $r(\mathbf{E}_c) = 5$. Too little information, a consistent case (a solution of the system (2) does exist but it is not unique; case 2(a)).

Biomass, ethanol, acetic acid: C = 5, $r(\mathbf{E}_c) = 5$. Sufficient information, a consistent case. The vector $\mathbf{n}_c$ can be calculated from eq. (4) (case 1(a)). In this case the first three columns of the matrix **E** in Table I constitute the matrix $\mathbf{E}_s$ and the remaining columns represent the matrix $\mathbf{E}_c$.

Let us consider that the following values of n_i were obtained by measuring: 0.350 mol for biomass, −1.089 mol for ethanol, and 0.0032 mol for acetic acid.[10] On substituting $\mathbf{E}_c$ and $\mathbf{E}_s$ into eq. (4), it is possible to calculate n_i for the remaining species, i.e., −1.815 mol for O_2, 0.831 mol for CO_2, −0.224 mol for NH_3, 2.372 mol for H_2O, and −2.450 mol for Ah.

These obtained results, however, are correct only if all the above assumptions are fulfilled (in particular, the knowledge of all the substances taking part in the fermentation process and absence of gross and systematic errors in the measurement of $\mathbf{n}_s$ values).

Biomass, ethanol, acetic acid, O_2, CO_2: C = 3, $r(\mathbf{E}_c) = 3$. Sufficient information, inconsistent case (too many specified species, case 1(b)). When we put, e.g., oxygen and carbon dioxide to those species, which are calculated, we obtain the preceding consistent case. If the vector $\mathbf{n}_s$ has been determined experimentally, it is possible to carry out a statistical analysis and smoothing of the measured values.[10] In this way it is possible to verify the validity of assumptions upon which the balance calculations are based.

DISCUSSION

Balance calculations are a useful tool in the design, optimization, and control of fermentation processes. They are based on the assumption that all the species taking part in the process of fermentation and significant with respect to the material balance are known. Our knowledge, however, may be rather inaccurate in this area. Problems may arise, e.g., from the biomass dissolved in the liquid phase, utilization

TABLE I
Atom Matrix **E**

Element	Biomass	EtOH	Acetic acid	O_2	CO_2	NH_3	H_2O	Mineral nutrients
C	3.83	2	2	0	1	0	0	0
H	7.00	6	4	0	0	3	2	0
O	1.94	1	2	2	2	0	1	0
N	0.64	0	0	0	0	1	0	0
Ah	7.00	0	0	0	0	0	0	1

of antifoaming agents by the microorganisms, formation of unknown fermentation by-products, etc. It is rather difficult to verify the validity of all assumptions upon which the balance calculations are based. The most reliable approach to the solution of this problem is a thorough statistical analysis of experimental data.[10] Such an analysis makes it possible to detect a number of unknown factors that must be taken into consideration when carrying out balance calculations.

Nomenclature

$\mathbf{A}$	matrix defined by eq. (4)
C	number of species calculated from material balance
e_{ji}	number of atoms E_j in species s_i
E_j	jth element
$\mathbf{E}$	atomic matrix $\{e_{ji}\}$
$\mathbf{E}_c$	atomic matrix of calculated species
$\mathbf{E}_s$	atomic matrix of specified species
I	number of species taking part in fermentation
J	number of elements E_j; rank of matrix $\mathbf{E}$
n_i	change in the number of moles of ith species due to fermentation
$\mathbf{n}_c$	vector of n_i values of calculated species
$\mathbf{n}$	vector of n_i values, $i = 1, \ldots, I$
$\mathbf{n}_s$	vector of specified values
$r(\ )$	rank of matrix
S	number of specified species
$\mathbf{W}_c$	covariance matrix of vector $\mathbf{n}_c$ errors
$\mathbf{W}_s$	covariance matrix of vector $\mathbf{n}_s$ errors

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