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STOICHIOMETRIC MODELLING FOR PHYSIOLOGICAL STATE CHANGES MONITORING IN BIOPROCESSES

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Abstract: This paper deals with the monitoring of physiological state changes that may occur during a culture of a single micro-organism. It rests on a partial measurement of component concentrations in the reactor and of the input and output flows and utilises a stoichiometric approach to model biochemical reactions involved by the culture. We propose to define the basic reaction structure from component compositions and from calculability conditions of the stoichiometric matrix. A given physiological state is associated with a set of basic reactions. The monitoring of a system is then derived from the obtained model by using a bank specialised unknown input observer. Copyright © 2004 IFAC

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1. INTRODUCTION

This paper deals with the monitoring of cultures that involve a single strain of micro-organism. The biological reactions that act in this process include microbial growth, maintenance and production reactions (Bastin and Dochain, 1990). In these types of reaction, substrates are consumed and are transformed either into biomass or into products.
The first modelling step is the definition of the biochemical reactions that are considered. In most of the proposed modelling approaches (Bastin, and Dochain, 1990, van der Hreijden, *et al.* 1993a, Dochain, and Perrier, 1997), this set is supposed to be *a priori* known and its design is not discussed. The dynamical model is derived from the reactions that are considered. It involves yield coefficients that have for a long time been introduced (Wang, *et al.*, 1977, Wang and Stephanopoulos, 1983, Tsai and Lee 1989,Bastin, and Dochain, 1990). Conditions of their identification from experimental data are settled in (Chen and Bastin, 1996) without discussing the influence of the reaction writing on this result.

This paper introduces the notion of basic reaction that ensures a single definition of the matrix of stoichiometric coefficients and thus the identification ability of its entries. As each basic reaction has a strong physiological meaning, their association can be used to define micro-organism physiological states. The environment - mainly the concentrations in various substrates with which the micro-organism is faced - often induces this state. The physiological state monitoring has previously been addressed. Konstantinov (Konstantinov and Yoshida, 1989) proposes a metabolic indicator based approach to govern the witching between metabolic states. The definition of these indicators rests on expert knowledge. In (van der Heijden, *et al.* 1993b, Shimizu, *et al.*, 1995), a single global reaction is concerned. An error vector is then calculated from the elementary balances that must be verified when this reaction actually acts. In (Takiguchi N., et al, 1997), the physiological state change from biomass

growth to lysine production is monitored on-line. It utilises a network of metabolic pathways based biochemical reactions to estimate lysine production from one-line measurements.

In this paper, an example, using the well-known yeast *Saccharomyces cerevisiae*, is used to illustrate all the concepts that are developed in this paper and that are discussed faced to works relative to the same topics. The dynamical model is quickly introduced. Then basic reactions definition is proposed from considerations on the yield coefficient matrix definition. Comparisons with other reactions definition and with yield coefficients identification conditions are developed. From the definition of physiological states as basic reactions subsets, a monitoring approach, derived from unknown input observer design, is proposed. A set of constraint equations to be verified by the measured conversion rates of relevant components is provided for each physiological state. Testing the residuals of these constraints leads to the recognition of the actual physiological state. The conclusion exhibits the interests of the proposed approach and some perspectives.

2. BASIC BIOCHEMICAL REACTIONS

2.1. Dynamic model

The fermentation in a bioreactor involves relevant components and micro-organisms. Regarding evolution of component concentrations in the liquid phase and microbial growth, the dynamic model rests on mass balance equations that are applied on each component present in the liquid phase. The biomass is here considered as a component. A dynamical model (Bastin and Dochain, 1990, Chen and Bastin, 1995) can be written as:

$$\frac{d}{dt}\xi = -D\xi + \mathbf{F} + \mathbf{Q}(\xi) + \mathbf{K}.\mathbf{r}(\xi) \qquad (1)$$

The n_p dynamical state vector ξ is the concentration vector of component involved by the reactions. In this model, the matrix $\mathbf{K} = \left[k_{ij}\right]$ is the matrix of stoichiometric coefficients of the reactions involved by the culture. The stoichiometric coefficients have to be distinguished from global conversion rates – or yield - that are further defined in part 3.4. By convention, entries of reaction rates $\mathbf{r}(\xi)$ are positive. Negative coefficients are thus associated with substrates in the reaction while positive ones are associated with product. A zeroed value indicates the component is not involved in the reaction (Chen and Bastin, 1995). D is the dilution factor and $\mathbf{F}$ and $\mathbf{Q}(\xi)$ are respectively liquid and gas rates of flow. The notation $\mathbf{Q}(\xi)$ here expresses the influence of the value of the continuous state vector ξ on exchange rate of flow between the gas the liquid phase. Evolution of the reaction rates $\mathbf{r}(\xi)$ is also function of this vector.

The sequel of this part aims at defining the $\mathbf{K}$ matrix. This definition rests on two main aspects: the definition of the reaction network structure – i.e. the components it involves that leads to coefficients different from zero – and the computation of these coefficient values.

2.2. Constraints on stoichiometric coefficients

The elementary composition of the components involved in the reactions is supposed to be known. Let $\mathbf{W} = \left[w_{kj}\right]$ be the composition matrix. Its entries w_{kj} give the elemental composition of the component j. The coefficient w_{kj} equals zero when atom of element k doesn't appear in the composition of the product j. $\dim(\mathbf{W}) = ne \times n$. ne is the number of different chemical elements that appear in the n component compositions. The constraints in terms of elemental balances must be fulfilled for each reaction. They can be written in the following relation form:

$$\mathbf{W}\mathbf{K} = \mathbf{0} \qquad (2)$$

for an illustration purpose, let us consider, the biomass X be *Saccharomyses cerevisiae*, glucose S, oxygen O_2, Ammonia NH_3 be substrates, carbon dioxyde CO_2, and water H_2O be products, ethanol E be either product or substrate. The composition matrix of these relevant components can be written using the compositions given in (Shimizu, *et al.*, 1995) choosing the orders X, S, O_2, CO_2, E, NH_3, H_2O for components and C H O N for chemical elements.

$$\mathbf{W} = \begin{vmatrix} 1 & 1 & 0 & 1 & 1 & 0 & 0 \\ 1.66 & 2 & 0 & 0 & 3 & 3 & 2 \\ 0.51 & 1 & 2 & 2 & 0.5 & 0 & 1 \\ 0.168 & 0 & 0 & 0 & 0 & 1 & 0 \end{vmatrix}$$

2.3. Basic reaction determination

The (bio)chemical reaction network involved in the fermentation is generally *a priori* defined from the physiological knowledge or from parameter identification from experiments. However, the writing of the reaction network is not unique. An inappropriate structure can lead to the inability to determine the coefficients of $\mathbf{K}$ as shown in (Chen and Bastin 1995). Let us introduce the notion of basic reaction.

Definition: A basic reaction is a reaction whose stoichiometric coefficients can be deduced in a single way from the constraint (2).

Without lost of generality, a normalisation component is selected for each reaction. Its corresponding stoichiometric coefficient is exactly fixed to one. The value of the r_i entry of $\mathbf{r}$ then gives the contribution of the reaction i to the conversion rates of its normalisation component.

Proposition 1: Let $\mathbf{W}_i$ *be the* $\mathbf{W}$ *matrix reduced to the* n_i *components involved by the reaction i.*

$$n_i - \text{rank}(\mathbf{W}_i) = 1$$

is a necessary and sufficient condition for the reaction i to be a basic reaction

Proof:
Relation (2) can be reordered according to the n_i components

$$\mathbf{W}\mathbf{K} = \begin{bmatrix} \mathbf{W}_i & \bar{\mathbf{W}}_i \end{bmatrix} \begin{bmatrix} \mathbf{K}_i \\ \mathbf{0} \end{bmatrix} = \mathbf{0} \qquad (3)$$

By definition, $\mathbf{K}_i$ must be a vector, as it corresponds to a single reaction. Relation (3) leads to:

$$\mathbf{W}_i \mathbf{K}_i = \mathbf{0} \qquad (4)$$

Thus $\mathbf{K}_i$ belongs to the left orthogonal subspace of the space spanned by the $\mathbf{W}_i$ matrix. As n_i corresponds to the $\mathbf{W}_i$ number of columns, $n_i - \text{rank}(\mathbf{W}_i)$ is the dimension of this subspace.Existence condition of $\mathbf{K}_i$ imposes this dimension to be strictly positive. The uniqueness of $\mathbf{K}_i$ direction imposes this dimension to equal 1. Indeed, let suppose the subspace dimension to be greater that one. $\mathbf{K}_i$ can then be any combination of the vectors of a basis $\mathbf{A}_i$ associated of this subspace and thus can't solely be defined. □

Proposition 2: The number of components involved in a basic reaction must not be greater than the number of chemical elements that appear in the compositions of these component plus 1.

Proof:
The proof rests on $\text{rank}(\mathbf{W}_i) \le ne_i$ where ne_i is the number of non-zeroed $\mathbf{W}_i$ rows. ne_i is the number of chemical elements involved by the composition of the components. The relation of the proposition 1 then becomes:

$$n_i - ne_i \le 1 \text{ and thus } n_i \le ne_i + 1 \square$$

Proposition 3: To provide a new basic reaction, the set of components of a basic reaction must not include the set of components of other basic reactions.

Proof:
If the set of components i includes a set j then $\mathbf{W}_i$ matrix includes a $\mathbf{W}_j$ matrix. Equation (4) becomes after an appropriate ordering of rows and columns:

$$\begin{bmatrix} \mathbf{W}_j & \mathbf{W}_k \end{bmatrix} \begin{bmatrix} \mathbf{K}_j \\ \mathbf{K}_k \end{bmatrix} = \mathbf{W}_j \mathbf{K}_j + \mathbf{W}_k \mathbf{K}_k = \mathbf{W}_k \mathbf{K}_k = \mathbf{0}$$

as $\mathbf{W}_j \mathbf{K}_j = \mathbf{0}$. $\mathbf{W}_k \mathbf{K}_k = \mathbf{0}$ can result from two possibilities : 1) $\mathbf{K}_k = \mathbf{0}$: The basic reaction is the basic reaction already defined and based on the set j of components 2) $\mathbf{W}_k \mathbf{K}_k = \mathbf{0}$: Then, two basics reactions based on sets j and k with fewer components than in the initial set can be generated and are thus preferred.□

Proposition 1, 2 and 3 provides us with rules that allows to select subsets of products, the admissible subset, that lead to basic reactions. The condition of proposition 2 only rests on matrix dimension considerations and can be easily checked. Generation of a set of reactions that satisfy the proposition 1,2 and 3 comes from as small as possible candidate subsets of components and from the selection of the reactions whose subsets are admissible. The main substrates are the guideline of this procedure that is given below.

Define the set of main substrates
Choose a main substrate.
Check whether a basic reaction can be found with this single substrate.
Iterate
  Generate subsets of admissible components by adding one of the remaining substrates excluding other main substrate
  Check whether a basic reaction can be found that involves a single main substrate.
Until the number of basic reactions equals the dimension of the $\mathbf{W}$ orthogonal space.

In our example, main substrate is glucose. The dimension of the $\mathbf{W}$ orthogonal space equals 3. The set of basic reactions that may be encountered during the *Saccharomyces cerevisiae* culture is given in Table 1, with a proposal for the physiological interpretation. It was previously proposed by (Liao, 1989) without justification of its writing. Applying conditions of propositions 2 and 3 leads for reaction (1) to 4 products for 3 elements and for reaction (3) to 5 products for 4 elements For reaction (2), $\text{rank}(\mathbf{W}_i) = ne_i - 1 = 2$. That lost of rank allows, using proposition 1, a reaction only involving 3 components for 3 chemical elements and a single substrate. These reactions are all the basic reactions

that can be generated using the glucose as main substrate.

Table 1 Basic reactions with glucose as substrate

$S + O_2 \rightarrow CO_2 + H_2O$ (1)
Respiration on glucose
$S \rightarrow CO_2 + E$ (2)
Ethanol production
$S + NH_3 \rightarrow X + CO_2 + H_2O$ (3)
Growth on glucose

2.4. Discussion

The interest of this definition of stoichiometric coefficient matrix is discussed now. Taking again our illustrative example, Table 2 exhibits reactions encountered during the *Saccharomyces cerevisiae* culture as they were proposed in (Dochain and Perrier, 1995). Let us check whether these reactions are basic reactions.

Table 2 Biochemical reactions supported by *Saccharomyces cerevisiae* from (Dochain and Perrier, 1995)

$S + O_2 \rightarrow X + CO_2$ (1a)
Respiratory growth on glucose
$S \rightarrow X + CO_2 + E$ (2a)
Reductive growth with ethanol production
$S + O_2 \rightarrow CO_2$ (3a)
Maintenance

All the reactions fulfil the condition of proposition 2. However, all the $\mathbf{W}_i$ matrices are square matrices with full rank and thus the existence conditions of proposition 1 are not fulfilled. Two components have to be added: one product, the water, and one substrate, ammonia. The modified reactions are given in Table 3.

Table 3 Modified reactions

$S + NH_3 + O_2 \rightarrow X + CO_2 + H_2O$ (1b)
$S + NH_3 \rightarrow X + CO_2 + E + H_2O$ (2b)
$S + O_2 \rightarrow CO_2 + H_2O$ (3b)

Reaction (3b) fit the conditions of propositions 2 and 1 and can then be kept as a basic reaction. The reactions (1b) and (2b) violate the propositions 2 and can't be basic reactions. However, the question of the equivalence of the representations has to be addressed. Reactions (1b) and (2b) can be seen as a combination of the 3 basic reactions as follows:
(1), (3) → (1b) and (2), (3) → (2b)
Thus, both writings allow describing the physiological reactions that act using glucose substrate. Ours provides a unique definition of stoichiometric coefficient matrix.

By definition basic reactions involve a minimum number of components. Using basic reactions thus lowers, from 13 to 9,the number of unknown coefficients that are necessary to define the **K**-matrix.

2.5. K-matrix calculation:

The calculation of the coefficients of **K** utilises directly the property of basic reaction: these coefficient are the entries of single normalised left null vector of the $\mathbf{W}_i$-matrices. In order to take into account ethanol as substrate two basic reactions have to be added. They are given in Table 4.

Table 4 Basic reactions with ethanol as substrate

$E + O_2 \rightarrow CO_2 + H_2O$ (4)
Respiration on ethanol
$E + NH_3 \rightarrow X + CO_2 + H_2O$ (5)
Growth on ethanol

Biomass, oxygen and ethanol are the normalised compound with this priority order. The **K**-matrix is then solely defined as:

	(1)	(2)	(3)	(4)	(5)
X	0	0	1	0	1
S	-1	-1.5	-1.034	0	0
O_2	-1	0	0	-1	0
CO_2	1	2	0.034	0.667	**-0.311**
E	0	1	0	-0.667	-0.689
NH_3	0	0	-0.168	0	-0.168
H_2O	1	0	0.456	1	0.456

The stoichiometric coefficient relative to CO_2 in reaction (5) is negative although this component is expected to be a product: this reaction must always be associated with a respiration reaction.

3. ON-LINE PHYSIOLOGICAL STATE MONITORING

3.1. Physiological state definition

The model (1) involves all the reactions that are known to appear during the culture. However, all these reactions don't occur simultaneously. The set of reactions that occur at a given time is induced by the physiological state of the micro-organism and by the availability of the substrates. A given physiological state is then defined by the set of reactions it involves. For the sake of simplicity, in the sequel of this paper, the set I of the active reactions will be used as a definition of the physiological state. The vector of the reaction rates $\mathbf{r}(\xi)$ is then reduced to a vector $\mathbf{r}^{(I)}(\xi)$ of lower dimension. In model (1), $\mathbf{K}^{(I)}$-matrix must be then substituted for the **K**-matrix. It gathers the columns of **K** associated to the active reactions in state I.

Table 5 Definition of physiological states

Physiological states	Involved reactions
I	(1)
II	(1) (3)
III	(2) (3)
IV	(1) (2) (3)
V	(4)
VI	(4) (5)

From the definition of basic reactions, determination of the states rests on choice of the main substrates that are used and on the oxygen availability. We suppose here that only one main substrate - glucose or ethanol - is used in a given physiological state. Physiological states that are then deduced from these principles can be found in Table 5. State IV both involves respiration and glucose reduction into ethanol. It is encountered when the yeast is faced with high glucose concentration and is referred as *Grabtree* effect.

3.2. Physiological state change detection

This part aims to determine how to detect a change in physiological states. Let us first consider the whole state ξ to be measured. The most realistic case of a partial measurement is developed in the next paragraph. Moving from a physiological state to another one corresponds to a change in the $\mathbf{K}^{(I)}$ matrix. Thus, the detection of a change in physiological state rests on the detection of a change in this matrix. For a given state, an appropriate unknown input observer can be constructed by projecting the state equations into a $\mathbf{K}^{(I)}$ orthogonal subspace. Let $\mathbf{R}^{(I)}$ be the projection matrix such that $\mathbf{R}^{(I)}.\mathbf{K}^{(I)} = 0$. The unknown input observer is written as:

$$\begin{aligned} \zeta^{(I)} &= \mathbf{R}^{(I)}.\xi \\ \frac{d\hat{\zeta}^{(I)}}{dt} &= -\mathbf{D}.\hat{\zeta}^{(I)} + \mathbf{R}^{(I)}.(\mathbf{F}+\mathbf{Q}) + \mathbf{G}.\varepsilon^{(I)} \\ \varepsilon^{(I)} &= \zeta^{(I)} - \hat{\zeta}^{(I)} \end{aligned} \qquad (5)$$

The $\mathbf{R}^{(I)}$ existence rests on the almost always-verified hypothesis that the number of active reactions is lower than the number of compounds they involve. Applying the observer (5) to the state equations (1) related to the state J leads to the dynamical equation of the observer output error.

$$\frac{d\varepsilon^{(I)}}{dt} = -(\mathbf{G}+\mathbf{DI}).\varepsilon^{(I)} + \mathbf{R}^{(I)}.\mathbf{K}^{(J)}.r^{(J)} \qquad (6)$$

When J=I, the output error is converging to zero if $\mathbf{G}$ has been designed in such a way that the $(\mathbf{G}+\mathbf{DI})$ matrix is stable. When the observer is no more matching the actual state J, $\varepsilon^{(I)}$ is the filtered image of $\mathbf{R}^{(I)}.\mathbf{K}^{(J)}.r^{(J)}$. The actual physiological state recognition will be performed from the $\varepsilon^{(I)}$ values that are governed by the terms $\mathbf{R}^{(I)}.\mathbf{K}^{(J)}$.

3.3. Monitoring with partial measurement

All the component concentrations can't be measured in (6). It is always true for water, and depends on the available instrumentation. When ξ is only partially measured, it can be split into $\xi = \begin{bmatrix}\xi_1 & \xi_2\end{bmatrix}^T$ where ξ_1 and ξ_2 are respectively the measured and unmeasured parts of the dynamical state vector. The measured part of (6) can be used. Using the measurable part of model (6) gives:

$$\frac{d}{dt}\xi_1 = -\mathbf{D}\xi_1 + \mathbf{F}_1 + \mathbf{Q}_1(\xi) + \mathbf{K}_1^{(I)}.r^{(I)}(\xi) \qquad (7)$$

An unknown input observer is designed by multiplying this model by a $\mathbf{R}_1^{(I)}$-matrix left orthogonal to $\mathbf{K}_1^{(I)}$. Choosing X, O_2, CO_2 as measured components leads to a matrix $\mathbf{K}_1$:

$$\mathbf{K}_1 = \begin{vmatrix} -1 & 0 & 0 & -1 & 0 \\ 1 & 0.5 & 0.034 & 0.667 & -0.311 \\ 0 & 0 & -0.168 & 0 & -0.168 \end{vmatrix}$$

Table 6 Relations to be verified in physiological states

state	Reactions		Constraints
I	(1)	-1 + RQ =0	[NH3] =0
II	(1) (3)	-1 + RQ -0.202*[NH3]/[O2] =0	
III	(2) (3)	[O2] =0	
IV	(1) (2) (3)		
V	(4)	-0.667 + RQ =0	[NH3] =0
VI	(4) (5)	-0.667+ RQ + 1.85*[NH3]/[O2] =0	

Table 6 gives the relations between the conversions rates $\mathbf{K}_1^{(I)}.r^{(I)}(\xi)$ of the measured components that are verified for each physiological state. No relation is found for physiological state (IV), as the associated matrix $\mathbf{K}_1^{(I)}$ is full ranked. The coefficients of these relations are deduced from the coefficients of $\mathbf{R}_1^{(I)}$ normalised according to the oxygen conversion rate [O2] to make appear the respiratory ratio RQ.

States all differ by at least one relation. That indicates the physiological states can be recognised from the result of the testing of these relations. In (Cassar, *et al.*, 2003), we discuss the conditions to be fulfilled to distinguish the state J from the state I. They depend on the ability to distinguish of the sets of reactions associated with I and J.

3.4. Discussion

The proposed approach has to be compared to the one that is presented in (van der Heijden *et al.* 1994). This paper deals with diagnosis and estimation of gross errors. It proposes three kinds of errors: Gross measurement errors, underestimated measurement noise and incorrect system definition. As the proposed method is not concerned with the two former errors, the comparison is focused on the last one. In this reference, the system definition rests on a single reaction involving a given set of components. The stiochiometry matrix is not explicitly defined as the conversion rate vector is concerned. Let $\mathbf{k}_r$ be this vector. It is directly deduced from the model (1) as: $\mathbf{k}_r = \mathbf{K}^{(I)}\mathbf{r}^{(I)}(\xi)$. As this vector is a variable linear combination of the column of $\mathbf{K}^{(I)}$ it verifies the elemental mass balance. The errors indicators are thus linear combination of residuals of the least square estimation, using the $\mathbf{W}^{(I)}$, of the measured $\mathbf{k}_r$ entries. Design of this linear combination is not discuss in the paper while, in our approach, it is derived from the basic reaction definition. Apparition of a new component conversion rate (ethanol by example from a pure respiratory growth) is seen as a composition or measurement error. An existing component conversion rate zeroing doesn't lead to an error and must thus to be checked especially. In our approach these two cases expressed by a physiological state change. However, conversion rate measurement errors are focussed on measurement error diagnosis and are better suited for this purpose.

4. CONCLUSION

In a first stage, we have proposed a definition of basic reactions that ensures the stoichiometric matrix to be uniquely defined. A constructive approach is proposed to derive a set of basic reactions from the compositions of components involved by the reactions. This systematic way of reaction network design avoids problems encountered with *a priori* design. As basic reactions involve a minimal set of components, they can be associated with elementary phenomena as respiration, glucose reduction into ethanol, biomass growth. In a second step, basics reactions are associated to define states whose physiological meaning is derived from the elementary phenomena they involve. An unknown input observer based method is proposed for the recognition of the actual physiological state from partial measurements. The design of such an observer using partial measurement shows the ability to monitor physiological changes. Some questions must still be studied thoroughly as estimation of the uncertainties and its introduction in the design of the decision process to test the constraints relative to each physiological state.

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