IFAC Publications
www.elsevier.com/locate/ifac

A HYBRID APPROACH FOR THE MONITORING OF PHYSIOLOGICAL STATE CHANGES.

J. Ph. Cassar, J. S. Guez, P. Jacques

LABEM,Polytech'Lille - USTL
Bd P. Langevin 596555 Villeneuve d'Ascq Cedex France
cassar@univ-lille.fr

Abstract: This paper deals with the monitoring of biological states that may occur during a culture of micro-organisms. A hybrid dynamic model is proposed. Each mode of the associated automaton is associated with a set of activated reactions which characterises a given biological state. The monitoring of a system described by such a model needs to evaluate the transition which is triggered on and when it is. The proposed solution deeply utilises the *a priori* knowledge. t is contained in the hybrid model, and doesn't need the specific growth rate estimation. It is developed in this paper considering that the whole state is measured and the matrix of the yield coefficients is known. *Copyright © 2003 IFAC*

Keywords: hybrid models, monitoring, biological application, physiological states.

1. INTRODUCTION

This paper deals with the modelling of reactions that involve a single strain of microorganism. The biological reactions that act in this process include microbial growth, maintenance and production reactions (Bastin and Dochin, 1990). In these types of reaction, substrates are consumed and are transformed into biomass, energy or products. Modelling of biological process is for a long time the purpose of many studies. This interest comes from the necessity of acquiring information about the behaviour of microorganism during the cultivation to better understand its dynamics. In the second hand, it intends to predict some information as microorganism or substrate concentration.

The approaches differ by the assumptions that are set about the reactions, by the knowledge that is available about the species concentration and the reactions.

In most of the proposed methods (Bastin, and Dochin, 1986, 1990, Dochin, and Perrier, 1997, Shimizu, *et al.*, 1989), one assumption is made about the set of reactions that is actually acting at each time. This set of reactions is closely related to the physiological state of the micro-organism. The environment - mainly the concentration in different substrates the micro-organism is faced with - often induces this state.

Hybrid approaches have already been proposed to deal with biological system. (Diaz, *et al.*, 1999) utilises this kind of representation in a multi-model monitoring of the specific growth rate function.

In (Alur, et al.,2002), a hybrid model is proposed to simulate - using the Charon programming language - the quorum sensing behaviour of a bacteria population. It models the transcription activation of the lux genes in the bacteria in the luminescence control process. The hybrid formulation of the model derives from the approximation of continuous function by piecewise constant functions.

The biological state monitoring has previously been adressed. In (Takiguchi N., et al, 1997), the physiological state change from biomass growth to lysine production is monitored on-line. In (Shimizu, *et al.*, 1995), a single global reaction is concerned. An error vector is then calculated from the elementary mass balances that must be verified when this reaction actually acts. A fuzzy classification procedure is utilised as in (Waissman, *et al.*, 1999) to identify the current physiological state. Konstantinov (Konstantinov and Yoshida, 1989) proposes a metabolic indicators based approach to govern the switching between metabolic states. The definition of the indicators rests on the expert knowledge.

Monitoring of hybrid system is less addressed. (Narasimhan S., *et al.*, 2000, McIlraith, *et al.*, 1999) propose dynamic simulation or observer to estimate the dynamic state and thus test transition conditions between the hybrid modes. (Cocquempot, et al. 2003) suggest testing the parity residuals deduced from the different dynamic models to achieve the same objective.

In the following paper, a hybrid modelling approach is proposed in which several physiological states are concerned. Each physiological state is associated with a set of activated reactions and corresponds to a logical state in the hybrid automaton. The definition of these states and the common dynamic behaviour are detailed in the third part. The definition of the invariant feature associated with each logical state is

then discussed. The practical testing of this invariant value is then applied.

An example (Dochin. and Perrier, 1997). using the well-known yeast *Saccharomyces cerevisae*. is used to illustrate all the concepts that are developed in this paper. The conclusion exhibits the interest of the proposed approach. the limitations it may be faced with and some perspectives.

2. DYNAMIC MODEL

The fermentation in a bioreactor involves products and microorganisms. Regarding the evolution of product concentration and microbial growth. the dynamic model rests on the balance equations that are applied on each product present in the liquid phase. The equations (1).(2) and (3) are respectively written for biomass X. for liquid substrates or products. and for gas substrates or products S under the assumption of perfect mixing in the stirred bioreactor.

$$\frac{dVX}{dt} = \sum_i k_{1i}.\rho_i - f_{out}X \qquad (1)$$

$$\frac{dVS_j}{dt} = \sum_i k_{ji}.\rho_i + \left(f_{in}S_{j,in} - f_{out}S_j\right) \qquad (2)$$

$$\frac{dVS_j}{dt} = \sum_i k_{ji}.\rho_i + q_j - f_{out}.S_j \qquad (3)$$

where f_{in} and f_{out} are respectively the input and output flows of the liquid phase. q_j is the gas flow of the element j exchanged with the liquid phase. V is the volume of the liquid phase. The variables X and S express the concentrations in biomass and in different molecules. The $k_{ji}.\rho_i$ terms represent the production or consumption rates of the element j related to the reaction i. $S_{j,in}$ is the j element concentration in the inflow liquid.

The liquid balance in the reactor is written as:

$$\frac{dV}{dt} = \left(f_{in} - f_{out}\right) \qquad (4)$$

Developing the derivative term in the left-hand side of the equation (1) to (3) and substituting (4) in these equations leads to the equations:

$$\frac{dX}{dt} = \sum_i k_{1i}.r_i - D.X \qquad (5)$$

$$\frac{dS_j}{dt} = -D.S_j + \sum_i k_{ji}.r_i + F_j \qquad (6)$$

$$\frac{dS_j}{dt} = -D.S_j + \sum_i k_{ji}.r_i + Q_j \qquad (7)$$

where $D = \frac{f_{in}}{V}$ is the dilution factor. F_j and Q_j the input flow relative to the volume respectively for the liquid and for the gas. The $k_{ji}.r_i$ terms represent the production or consumption rates relatively to a unit volume. Writing the equations (5) to (7) into a vectors and matrices form leads to the general model (Bastin and Dochin. 1990):

$$\frac{d}{dt}\xi = -D\xi + \mathbf{F} + \mathbf{Q}(\xi) + \mathbf{K}.\mathbf{r}(\xi) \qquad (8)$$

The notation $\mathbf{Q}(\xi)$ expresses here the influence of the value of the continuous state vector ξ on flow exchange rate between the gas and the liquid phase. The evolutions of the production or consumption rates $\mathbf{r}(\xi)$ are also functions of this vector.

3. HYBRID APPROACH

This part presents a dynamic hybrid automaton based approach for the dynamic system model (8) when physiological state changes occur. The formal description of hybrid models has extensively been described and will not be recalled here. Such a description can be found in (Alur R., *et al*,. 1995, Boel R.K., *et al*., 1999, Zaytoon, 2001). Most of this formalism is needed for simulation or stability study. The present approach will only keep the following notions: location or modes and their associated dynamic model. transitions. guard on the transitions. The biological motivation of this kind of model is first discussed.

3.1. Biological motivations

The hybrid modelling is motivated by the fact that all the reactions can't act simultaneously. Then the vector $\mathbf{r}$ possesses at each instant a particular structure in which non-active reactions correspond to zero.

The activation of a given reaction verifies some necessary conditions that can be expressed as the availability of the substrates consumed by the reaction. These constraints can be expresses as:

$$\mathbf{C}_i^+.\xi > \varepsilon_i \qquad (9)$$

where $\mathbf{C}_i^+$ is the selection matrix of the substrates that are needed by the reaction i. ε_i is the vector of minimal concentrations required for the reaction activation. Table 1 exhibits an example of biological reactions encountered during the *Saccharomyces cerevisae* culture (Dochin and Perrier. 1995).

The state vector in this example contains the concentrations of the species involved in the reactions: $\xi = \left[X \quad S \quad O_2 \quad CO_2 \quad E\right]^T$. The concentrations are respectively the biomass. glucose. oxygen. carbon dioxide and ethanol concentrations.

Reaction (2) needs a S positive concentration and the constraint (9) is given by :

$$\left[0 \quad 1 \quad 0 \quad 0 \quad 0\right].\xi > \mathbf{0}.$$

However. reaction (3) is inhibited when glucose

Table 1 Biological reactions supported by *Saccharomyces cerevisae*

$S + O_2 \rightarrow X + CO_2$	(1)	**Respiratory growth on glucose**
$S \rightarrow X + CO_2 + E$	(2)	**Reductive growth with ethanol production**
$E + O_2 \rightarrow X + CO_2$	(3)	**Respiratory growth on ethanol**
$S + O_2 \rightarrow CO_2$	(4)	**maintenance**

concentration is over a given small value. A new type of constraint applied on the state value is then derived:

$$\mathbf{C}_i^0 \cdot \boldsymbol{\xi} < \boldsymbol{\varepsilon}_i \qquad (10)$$

A given set of reactions is said compatible when the set of constraints (9) and (10) associated with it can be satisfied.

3.2. Associated hybrid automaton

The compatible reactions sets lead to define the nodes of the hybrid automaton associated with a physiological state (here called modes). The constraints constitute then the invariant associated with each node. For the node I, it can be written in a general form that includes both constraints (9) and (10):

$$\mathbf{C}_i(\boldsymbol{\xi}, \boldsymbol{\varepsilon}) > 0 \qquad (11)$$

Transitions between the nodes result from the continuous state evolution that is governed by the dynamic model (8). In this expression, specie consumption is traduced by a negative term in the K matrix while production is expressed by a positive component (see Table 2).

A transition occurs when one of the constraints that are associated with the current mode is no more satisfied. That can result from two kinds of evolution. In the first kind, the state might evolve to a constraint firing without any action on the command variables **F** and **Q**. These transition are called autonomous transitions. In another hand, for instance when a constraint of type (10) is verified and when the associated specie can't be produced by one of the active reactions, the evolution of the associated concentration can only occur when an action on the command variables is applied.

Applying these principles to the reactions of the Table 1, leads to the hybrid automaton of Figure 1. In this figure, S,O,E express respectively : S, O_2, and Ethanol concentrations.

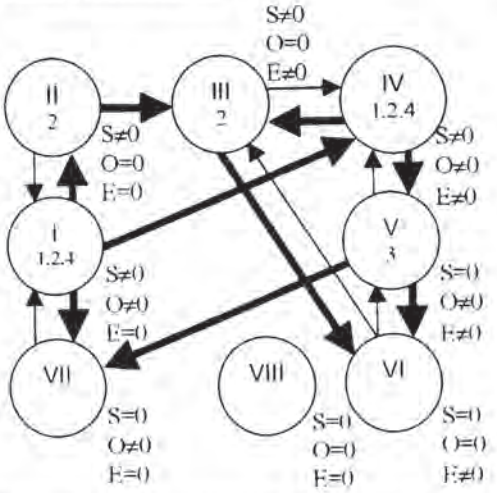

Figure 1 Hybrid automaton associated with the Table 1 reactions. The bold arrows indicate autonomous transitions while the other ones depend on an external action. The activated reactions linked with a given mode are listed below the mode number.

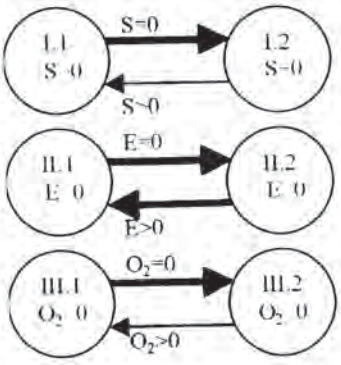

Figure 2 Elementary automaton. Equalities and inequalities correspond respectively to constraints (9) and (10).

The mode II is a transient mode as reaction (2) produces ethanol and then the condition E=0 can't be satisfied for a long time.

When the number of species is growing the description of the automaton can't be performed as in Figure 1. Indeed the number of nodes results from the combination of the constraints that increase very fast with the number of species. If only two states are associated with each concentration (zero, non zero), then 2^n combinations are possible (n being the number of species). In order to face this problem, the hybrid automaton can be derived from the product of very simple automaton, associated with the evolution of each substrate, as presented in the Figure 2.

Table 2 gives the link between the modes and the conditions for the activation of each reaction. It can be noticed that a fifth reaction must bee added that expresses the mortality. Indeed the lack of several species doesn't allow any reaction to be active and in these cases the micro-organisms can't maintain themselves and then die. This reaction is associated with the combinations of state I.2&III.2 (state VI in the hybrid automaton of Figure 1) and I.2&II.1 (state VII). The state corresponding to the lack of three substrates (state VIII in figure 1) can only be reached in some few particular conditions. Indeed, in states VI and VII, no reaction can consume the remaining substrate.

The transitions in Figure 2 are not always allowed if the correspondence with the automaton of Figure 1 is to be obtained. Some guards are then defined that restrict the possibility of the spontaneous transitions. They are based on the following principle: a spontaneous transition can only be validated if guard

Table 2 Elements of the K matrix and relations between reaction and the allowed states

	(1)	(2)	(3)	(4)	
X	1	1	1	0	-1
S	$-k_{21}$	$-k_{22}$	0	-1	0
O_2	$-k_{31}$	0	$-k_{33}$	$-k_{34}$	0
CO_2	k_{41}	k_{42}	k_{43}	k_{44}	0
E	0	k_{52}	$-k_{53}$	0	0
	I.1 III.1	I.1	I.2 III.1	II.2 I.1 III.1	

Table 3 Guards applied on the Figure 2 transitions

Transition	Guards	reactions
$E=0$	I.2 AND III.1	(3)
$E>0$	I.1	(2)
$O_2=0$	II.1 OR II.2	(1) OR (4)

conditions are fulfilled. Transition guards to be applied on Figure 2 to obtain hybrid automaton of Figure 1 are given in Table 3. They express the need of the activation of the reaction that leads to the substrate consumption or production.

Figure 1 is good tool for studying the possible evolution of the cultivation and the cycles that can occur in it. The hybrid model can thus be utilised as a tool for the *a priori* process analysis. It can also be used for a monitoring purpose.

3.3. Monitoring use

If transition conditions can be tested, the proposed hybrid model allows following the evolution of the biological state of the culture. Testing the transition condition can be performed on the constraint form (11). This solution is proposed in the few papers that deal with the supervision of dynamic hybrid systems (Narasimhan S., *et al.*, 2000, McIlraith, *et al.*, 1999) However, these methods need the definition of the limits ε that may be hardly established. As this work aims at monitoring new biological processes and may need the elaboration of hybrid model during the experiments, the test of the transition conditions must rest on other principles. When a transition is triggered on, the concentration values allow establishing an estimation of the limits of the invariant set. This utilisation of hybrid model to follow the biological states is original in the hybrid framework. Indeed, most of the approaches consider the invariant definition as *a priori* known and are not concerned with their estimation (Narasimhan S., *et al.*, 2000, McIlraith, *et al.*, 1999).

4. ON-LINE BIOLOGICAL STATE MONITORING

This part aims to determine how to detect a change in the hybrid system mode. The matrix $\mathbf{K}$ is supposed to be known. For example, it can be derived from considerations about the stoichiometric relations or from a previous learning stage (Chen and Bastin, 96). The whole state ξ is supposed to be measured.

4.1. Changing mode detection

The model (8) can be written for each mode I of the hybrid model. In this case, a matrix $\mathbf{K}^{(I)}$ is substituted for the matrix $\mathbf{K}$. $\mathbf{K}^{(I)}$ matrix gathers the columns of $\mathbf{K}$ associated with the active reactions in mode I.

Moving from a mode to another one corresponds to a change in the $\mathbf{K}^{(I)}$ matrix. Thus, the detection of a change in mode rests on the detection of a change in the $\mathbf{K}^{(I)}$ matrix.

At a given mode, an appropriate unknown input observer can be constructed by projecting the state equations into a $\mathbf{K}^{(I)}$ orthogonal subspace. Let $\mathbf{W}^{(I)}$ be the projection matrix such that $\mathbf{W}^{(I)}.\mathbf{K}^{(I)}=0$. The unknown input observer is written as:

$$\begin{aligned}\zeta^{(I)} &= \mathbf{W}^{(I)}.\xi \\ \frac{d\hat{\zeta}^{(I)}}{dt} &= -D.\hat{\zeta}^{(I)} + \mathbf{W}^{(I)}.(\mathbf{F}+\mathbf{Q}) + \mathbf{G}.\varepsilon^{(I)} \\ \varepsilon^{(I)} &= \zeta^{(I)} - \hat{\zeta}^{(I)}\end{aligned} \quad (12)$$

The $\mathbf{W}^{(I)}$ existence rests on the almost always verified hypothesis that the number of active reactions is lower than the number of species they involve (Chen, *et al.*, 2000).

Applying the observer (12) to the state equations (8) related to the mode J leads to the dynamic equation of the observer output error.

$$\frac{d\varepsilon^{(I)}}{dt} = -(\mathbf{G}+\mathbf{DI}).\varepsilon^{(I)} + \mathbf{W}^{(I)}.\mathbf{K}^{(J)}.\mathbf{r}^{(J)} \quad (13)$$

When the modes related to the observer and the actual reactions are corresponding – i.e. J=I – then 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 mode J, a change in mode must be decided. In this case, $\varepsilon^{(I)}$ is the filtered image of $\mathbf{W}^{(I)}.\mathbf{K}^{(J)}.\mathbf{r}^{(J)}$.

The transition triggering on will be decided from the $\varepsilon^{(I)}$ value that is governed by the term $\mathbf{W}^{(I)}.\mathbf{K}^{(J)}$. This term must then be more carefully examined.

Let R(I) be the set of reactions involved by the mode I (in the Figure 1, for instance, R(IV)={1,3,4}) and let $\mathbf{K}^{(i)}$ be the column of $\mathbf{K}$ associated with the reaction i.

$\mathbf{W}^{(I)}$ is defined by the condition $\mathbf{W}^{(I)}.\mathbf{K}^{(I)}=\mathbf{0}$ that can be written as :

$$\mathbf{W}^{(I)}.\mathbf{K}^{(i)}=\mathbf{0} \quad \forall i \in R(I) \quad (14)$$

Two cases are then to be considered that will allow deriving the appropriate rule for transition triggering.

In the first case, $R(J) \subset R(I)$. From the condition (14), it can be deduced that:

$$\mathbf{W}^{(I)}.\mathbf{K}^{(j)}=\mathbf{0} \quad \forall j \in R(J) \quad (15)$$

The condition (15) is equivalent to $\mathbf{W}^{(I)}.\mathbf{K}^{(J)}=\mathbf{0}$.

In this case, the mode change from I to J doesn't lead $\varepsilon^{(I)}$ to differ from zero. In the proposed example, the transition from mode I to mode II only corresponds to this configuration.

The alternate case, $R(J) \not\subset R(I)$ is the most general case. It occurs when the set of reactions associated with the mode J is not included in the set of reactions associated with the mode I.

$$\mathbf{W}^{(I)}.\mathbf{K}^{(j)} \neq \mathbf{0} \quad \forall j \in R(J) \setminus R(I) \quad (16)$$

where $R(J) \setminus R(I)$ is the complementary set of R(I) in R(J). It can be deduced from (16) that $\mathbf{W}^{(I)}.\mathbf{K}^{(J)} \neq \mathbf{0}$. Thus for $\mathbf{r}^{(J)} \neq \mathbf{0}$, that is supposed

to be verified as the reactions of mode (J) are active. $\varepsilon^{(I)} = \mathbf{W}^{(I)}\mathbf{K}^{(J)}\mathbf{r}^{(J)}$ differs from zero and its norm can be tested to evaluate a change from the mode I.
These both cases can be closely related to the concept of discernability between modes proposed in (Cocquempot, et al., 2003. Two modes (I) and (J) can be discerned if, for at least one of the two modes, the indicator associated with this mode is triggered on when computed by using data from the system operating in the other mode.

4.2. Evaluation of the transition to be activated

Let S(I) be the set of the successors of I in the hybrid graph (for example, S(IV) = {III,V} while S(II)={III}).
We first consider $S^+(I)$ the set of the successors that satisfy the second case of the former paragraph.
The evolution rule in the hybrid automaton can be settle as :

$$I^+ = \arg\min_{K \in S^+(I)} \left(\left\| \varepsilon^{(K)} \right\| \right) \qquad (17)$$

I^+ is the value of the next mode when the $\varepsilon^{(I)}$ norm is different from zero. "argmin" provides the mode that corresponds to the minimum norm value. This rule corresponds to a multi-model approach and is only activated when the test applied on $\varepsilon^{(I)}$ is triggered on. The definition of the hybrid automaton permits to limit the number of simultaneous models to be tested. It is restricted to the number of the current mode successors.
Dealing with the successors corresponding to the first case needs to activate two observers in the same time that give the values of $\varepsilon^{(I)}$ and $\varepsilon^{(J)}$.
When I is the actual mode, $\mathbf{W}^{(J)}\mathbf{K}^{(I)}$ differs from zero as R(I) can't be included in the set R(J) and thus $\varepsilon^{(J)}$ differs from zero. The different combinations of observers and the modes are summarised in the Table 4.

Table 4. Values of observer outputs

		Actual mode I	Actual mode J
Observer outputs	$\varepsilon^{(I)}$	0	0
	$\varepsilon^{(J)}$	1	0

In this table, the zeroed values indicate that the norm of the observer output vector is expected to be near to zero while the "1" value expresses that the associated output must differ significantly from zero. Using the two observers output vector and testing their norm to zero allows distinguishing mode J from mode I. The transition from I to J is triggered on when the observer output associated to J comes to zero.
Applying these two rules and adapting the observers to current mode allows then following the evolution of the current mode.

5. APPLICATION

To illustrate the results given above, the table 5 exhibits the **K** matrix for the reactions of table 1. This matrix has been computed to be orthogonal to the elementary composition matrix of the products involved in the reactions. Some products were added to allow the K computation such as water or NH_3. Only the part of K corresponding to the products considered above is given here. It can be noticed that the writing of the reactions (1) and (2) does not lead to a single vector to describe the generated space. In another hand, the structure of reaction (2) has been modified because the structure of table 1 doesn't give any solution for the corresponding column of **K**. The biological meaning of the reaction is then changed. Taking into account the consistency of the set of reactions is out of the topic of this paper and can not be more detailed.
Table 6 gives the value of the projection matrices $\mathbf{W}^{(I)}$ orthogonal to the $\mathbf{K}^{(I)}$ matrices corresponding to the mode (I). The dimension of the generated space is equal to $\left(5 - \text{rank}\left(\mathbf{K}^{(I)}\right)\right)$.

Table 5. Value of **K** matrice

	Reactions 1		2		3	4
Products						
X	1	1	0	1	1	0
S	-0.67	16.17	-1	0	0	-1
0_2	0.36	17.21	0	0.698	-0.62	1
CO_2	-0.33	-17.17	0.33	-0.776	0.10	1
E	0	0	0.66	-0.223	-1.10	0

Table 6. Values of orthogonal matrices related to modes

$\mathbf{W}^{(I)}$	0.3931	0.3753	-0.5249	-0.1496	0.6377
$\mathbf{W}^{(IV)}$	0.3252	0.3396	0.4239	0.7635	0.1277
$\mathbf{W}^{(II)}$	0.8018	0.3571	0	0.2143	0.4286
$\mathbf{W}^{(III)}$	0	0	1	0	0
	-0.2673	0.2143	0	0.9286	-0.1429
	-0.5345	0.4286	0	-0.1429	0.7143
$\mathbf{W}^{(V)}$	-0.1861	0.6809	0.4836	0.3140	-0.4114
	0.3702	-0.3750	0.3603	0.7427	0.2023
	0.4221	0.6290	-0.3087	0.1029	0.5659

Table 7. Expected observer ouputs

	Actual reactions 1		2		3	4
Observers						
(I)	0	0	0	0	0	0
(IV)	0	0	0	0	0	0
(II)	1	1	0	1	1	1
(III)	1	1	0	1	1	1
	1	1	0	1	1	1
	1	1	0	1	1	1
(V)	1	1	1	0	0	1
	1	1	1	0	0	1
	1	1	1	0	0	1

Table 7 results from the projection of **K** into the subspace associated with the modes. The result has been fixed to 0 when the component equals zero and 1 everywhere else. This table clearly shows that projection matrix linked with modes (I) and (IV) is also orthogonal to matrices $\mathbf{K}^{(II)} = \mathbf{K}^{(III)}$ and $\mathbf{K}^{(V)}$. The first result is expected as $R^{(II)} = R^{(III)} = \{2\}$ is included in $R^{(I)} = R^{(IV)} = \{1,2,4\}$. However the second result indicates that the columns of $\mathbf{K}^{(V)}$ belongs the subspace generated by $\mathbf{K}^{(I)} = \mathbf{K}^{(IV)}$.
However, the three sets of modes can be distinguished. Indeed, they meet the dicernability condition, as the outputs of the unknown input observers are different according to the actual active mode. That imposes, in mode I and IV, running the three observers while the other modes only need a single observer. A hybrid model can thus monitor the system evolution.

6. CONCLUSION

In this paper, we have proposed a first approach to model the evolution of the biological states during a microorganism cultivation. It differs from other solutions that consider all the reactions are be together activated and thus all the products are together involved. The production or consumption rates are then to be estimated and evaluated to determine the active biological state. It can be noticed that the reduced rate vector value can be estimated more accurately from the reduced matrix $\mathbf{K}^{(I)}$. Some questions must still be studied thoroughly - as the estimation of the transition time, the use of a partial state measure, partial knowledge about **K** ,- to give this work a wider range of application.

REFERENCES

Alur R., Belta C., Kumar V., Mintz M., Pappas G. J., Rubin H., Schug J. (2002). Modeling and analyzing Biomolecular Networks. *Computing in Science & Engineering*, , 2-13

Alur R., et al. (1995). The algorithmic analysis of hybrid systems. *Theoretical Computer Science*, **138**, 3-34

Bastin G., Dochain D. (1990). On line estimation and adaptative control of bioreactors. Elsevier, Amsterdam

Bastin G., Dochain D. (1986). On-line estimation of microbial specific growth rates. *Automatica*, **22-6**, 705-709

Boel R.K., et al. (1999). Approaches to Modelling, Analysis and Control of Hybrid Systems. *Journal A*, **40-** 4, 16-27

Chen L., G. Bastin (1996). Structural Identifiability of the Yield Coefficients in Bioprocess Models when Reaction Rates are Unknown. *Mathematical Biosciences*, **132**, 35-67

Cocquempot V., Staroswiecki M., El Mezzyani T. (2003). Switching Time Estimation and fault detection for Hybrid Systems Using Structured Parity Residuals. *IFAC Conference Safeprocess 2003*, Washington, DC, USA.

Diaz. C., P. Lelong, P. Dieu, C. Feuillerat, M. Salomé (1999). On-line analysis and modeling of microbial groth using a hybrid system approach. *Process Biochimie*, **34**, 39-47

Dochain D., M. Perier (1997). Dynamical Modelling, Analysis, Monitoring and Control Design for Non Linear Bioprocesses. *Biotechnology*, **56**, 147-197

Konstantinov K., T. Yoshida (1989). Physiological State Control of Fermentation Processes. *Biotechnology and Bioengineering*, **33**, 1145-1156

McIlraith, S., Biswas, G., Clancy, D., Gupta, V. (June 1999). Towards diagnosing Hybrid Systems. *Proceedings of the Tenth International Workshop on Principles of Diagnosis (DX'99)*, Loch Awe Scotland, 193-203

Narasimhan S., F. Zhao, G. Biswas, and E. Hung (2000). Fault Isolation in Hybrid Systems Combining Model-based Diagnosis and Signal Processing. *4th Symposium on Fault Detection, Supervision, and Safety Processes*, Budapest Hungary, 1074-1079

Narasimhan S., G. Biswas, G. Karsai, T. Pasternak, and F. Zhao (2000). Building Observers to Handle Fault Isolation and Control Problems in Hybrid Systems. *Proc. 2000 IEEE Int. Conference on Systems, Man, and Cybernetics*, Nashville TN, 2393-2398

Shimizu H., & al. (1989). An Algorithmic Approach to Constructing the on-line Estimation System for the Specific Growth Rate. *Biotechnologigy and Bioengineering*, **33**, 354-364

Shimizu H., K. Miura, S. Suteaki, K. Suga (1995). On-Line State Recognition in a Yeast Fed-batch Culture Using Error Vectors. *Biotechnology and Bioengineering*, **47**, 165-173

Takiguchi N., & al (1997). On-line Physiological State Recognition System for the Lysine Fermentation Process Based on a Metabolic Reaction Model. *Biotechnology and Bioengineering*, **55-** 1, 170-181

Waissman J, Pourciel J. B. , Roux G. , Dahhou B. , Goma G (1999). La fermentation automatisée. *Biofutur*, **187**

Zaytoon J. (2001). Systèmes dynamiques hybrides. Hermès, Paris France