Supervision, Control and Optimization of Biotechnological Processes

Based on Hybrid Models

Dissertation

zur Erlangung des akademischen Grades

Doktor-Ingenieur (Dr.-Ing.)

vorgelegt der

Von der Mathematisch-Naturwissenschaftlich-Technischen Fakultät

- Fachbereich Verfahrenstechnik -

der Martin-Luther-Universität Halle-Wittenberg

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geb. am 31. Mai 1969 in Fafe/Portugal

Acknowledgements

I acknowledge Angelika and Wilhelm for the most friendly "Willkommen in Deutchland". They were my first contact in Germany and, as matter of fact, it revealed itself to be a very pleasant first contact. They assisted me on learning German and many other things about Germany, including the best bier, the best food, and several interesting places to visit.

I acknowledge Traudel and Herbert for the weekends in Gronau, with marvellous meals, and always friendly receptions. This was the ideal environment for fighting many stress situations. I acknowledge them very specially for the warm receptions on Christmas.

I acknowledge all my work group colleges in Portugal in FEUP and UM: Antonio Ferreira for the help on almost everything concerning software, hardware and programming, Eugenio Ferreira for important tips about adaptive systems theory, Filomena Oliveira for the know-how on baker's yeast cultivation, Margarida for the tips on optimization, and very specially Joana for the enormous sympathy.

I acknowledge all my work group colleges in Germany in TCI and MLU. There are lots of people I have to acknowledge. I'll begin with Martin Manikowski just by saying that he was a great college and is one of the best friends I ever had. I acknowledge Dieter for the support he gave me in many difficult life situations, what he did and still does in a way that only very good friends can do, and of course, for the baker's yeast data. I acknowledge our NT specialist, Stephan Klusmann, who between many things, helped a lot to improve my social life in Germany. I acknowledge Dr. Lapin and Dr. Simutis for the excellent scientific support. They just know everything about computers and biotechnology. To work with them was an honour. I acknowledge Franco for the big help in developing HYBNET's help system. I acknowledge Dr. Havlik and Dr. Dors for the hardware and software support. I acknowledge Wolfgang Claas mainly for the good disposition. I acknowledge Dr. Volk for the help in all the preliminary paper work at the MLU for the present PhD work.

I acknowledge Prof. S. Feyo de Azevedo and Prof. A. Lübbert for the scientific supervision. They gave me a very positive orientation in many different aspects. Both of them took an active role on developing the present work for what I most sincerely acknowledge.

I acknowledge Anke just for the help on almost every aspects of my life in Germany. She even took an active role on typing this thesis because I broke my hand one and a half month before the deadline.

I acknowledge very specially my Mother and my Father for the given opportunity. They never doubted that this was a deserved opportunity. I acknowledge them most sincerely for the given financial support.

I dedicate my thesis, which represents 4 years of my life of hard work, to my daughter Linn Philomena. She had the magic to make me forget all the problems just by smiling. Problems that seemed impossible to solve few moments before. She was and she is a reason to live to be happy and to keep fighting. Whatever it happens in the future, I'll keep it this way.
THANK YOU LINN!

Summary

Process optimisation, supervision and control are becoming increasingly important issues due to hard competition between companies. Still, the acceptance and implementation of model-based methodologies for process improvement remain rather limited in the industry, mainly because the benefit/cost ratio is not yet clearly attractive for such developments in process operation. The main goal of the present PhD thesis is to develop methodologies to improve this benefit/cost ratio in the industrial practice.

Biochemical processes are very complex and often poorly understood on a mechanistic basis, particularly in what concerns the microorganisms growth mechanisms. In the classical approach for process analysis mathematical models, based on first principles, are used to represent the mechanistic *a priori* knowledge about the process in study. Most often this approach leads to complex models made of poorly understood mechanisms, the latter characterised by parameters with a very low level of confidence. It may lead to poor process description and have too high development costs involved.

The main subject of this work concerns the search and study of methodologies for integrated use of all available *a priori* process knowledge, from mechanistic to heuristic knowledge, aiming at a more accurate process description with lower development costs. In this respect, the hybrid modelling approach has been extensively studied and developed. In particular, a new approach for hybrid modelling was developed based on the concept of hybrid networks. Hybrid networks provide means to incorporate arbitrarily in one computational structure all sources of *a priori* knowledge in the levels of sophistication available in practice. They can be used for process identification, on-line and off-line process optimisation, process control and process supervision. They have the additional very attractive property that the backpropagation technique can be applied to support parameter identification and sensitivity analyses. A relevant result was the development of the HYBNET software package, which implements the concept of HYBrid NETworks. The software was designed to provide all the necessary tools to solve typical tasks of process optimisation and control usually found in the industry. A very important goal was the development of a nearly platform independent and easy to implement link between hybrid network based algorithms and the process. This, jointly with an user-friendly graphical interface, are recognised to be decisive pre-requisites for a good acceptance in the industrial environment.

The other important issue studied in the present work was the problem of efficient and rational use of process information on-line. When on-line information is available, it is possible to use a compromising solution, employing algorithms based in simplified models, complemented with on-line adaptation schemes. Such a compromising approach is presented here. The development of models assuming no knowledge about the microorganisms growth kinetics is rather simple and, consequently, effort was put on developing strategies for on-line estimation of reaction kinetics from data available on-line. With this respect two stable and easy to tune on-line reaction rates estimation algorithms have been developed. They explore the relationships between stability and dynamics of convergence, imposing convenient second-order trajectories for the estimation errors. Tuning requires only the setting of the parameters characteristic of second-order responses - the damping coefficient and the natural period of oscillation. This represents a lower development cost than the cost of the usual 'trial and error' techniques employed in the daily practice of industrial process operation.

Zusammenfassung

Prozeßoptimierung, Überwachung und Kontrolle wird durch den harten industriellen Wettbewerb immer wichtiger. Trotzdem bleibt die Akzeptanz einer Prozeßoptimierung mit Hilfe von Modellen in der Industrie gering, vor allem wegen ihrer zu kleinen Gewinn-/Kosten-Verhältnisse. Das Hauptziel der vorliegenden Arbeit ist, Methoden zu entwickeln, um das Verhältnis von Gewinn zu Kosten in der industriellen Produktiß zu verbessern. Biochemische Prozesse sind sehr komplex und i.a. in mechanistischer Hinsicht ungenügend aufgeklärt und verstanden. Beim klassischen Weg der Prozeßverbesserung repräsentieren mathematische Modelle das mechanistische *a priori* Wissen des zu untersuchenden Prozesses. Die Folge davon ist, daß viele dieser Modelle den Prozeßverlauf ungenügend darstellen und zu hohe Entwicklungskosten implizieren. Das effiziente Nutzen des vorhandenen *a priori* Wissens ist ein Hauptthema dieser Arbeit. Die Nutzung aller vorhandenen -mechanistischen und heuristischen- Wissensquellen führt zu einer genaueren Prozeßbeschreibung und niedrigeren Entwicklungskosten. Diese Methode der Hybrid-Modellierung wurde extensiv untersucht und entwickelt. Insbesondere eine neue Methode der Hybrid-Modellierung, basierend auf dem Konzept eines hybriden Netzwerkes, wurde entwickelt. Hybride Netze liefern die Möglichkeit der arbiträren Integration aller zur Verfügung stellenden Quellen des *a priori* Wissens in einer Modellstruktur. Sie können zur Prozeßidentifizierung, *on-line* und *off-line* Prozeßoptimierung, -kontrolle und -überwachung genutzt werden. Zusätzlich haben sie die attraktive Eigenschaft, daß die Rückkopplungstechnik angewandt werden kann, um die Parameteridentifizierung und Empfindlichkeitsanalysen zu unterstützen. Ein weiteres wichtiges Resultat war die Entwicklung des HYBNET-Software Pakets, das das HYBride NETzwerk einsetzt. Die Software ist so entworfen worden, daß sie alle nötigen Instrumente zur Bearbeitung von Aufgaben der Prozeßoptimierung und -kontrolle für die Industrie liefert. Ein sehr wichtiges Ziel war die Entwicklung einer quasi plattformunabhängige und einer leicht einsetzbaren Verbindung zwischen dem Hybriden Netzwerk und dem Prozeß. Dies, zusammen mit einer benutzerfreundlichen graphischen Oberfläche, ist ausschlaggebend für die Akzeptanz in der Industrie. Mit HYBNET sind die Aufgaben der *on-line* Ausführung Hybrider Netzewerk wesentlich vereinfacht worden. Ein anderes wichtiges Ergebnis, das während der vorliegenden Arbeit untersucht wurde und im engen Zusammenhang mit den Kosten einer Prozeßverbesserung durch Modellen steht, ist das Problem einer effizienten und rationalen Nutzung der *on-line* Prozeßinformation. Stehen *on-line* Prozeßinformationen zur Verfügung, so besteht die Möglichkeit auf einfachen Modellen basierende Algorithmen zu nutzen, ergänzt mit *on-line* Anpassungsalgorithmen. Dies ist ein relevantes Problem der Prozeßverbesserung mit Hilfe von Modellen: Entweder mehr Mittel in die Prozeßmodellierung oder in die Entwicklung robuster und stabiler *on-line* Anpassungsalgorithmen zu investieren. Das Optimum ist ein Kompromiß zwischen beiden Entwicklungen. Modelle, die die Wachstumskinetik der Mikroorganismen nicht berücksichtigen, sind besonders simpel. Es wurde viel Wert auf die Entwicklung von Strategien zur *on-line* Abschätzung der Reaktionskinetiken aus den vorhandenen *on-line* Daten gelegt. Es wurden zwei stabile und leicht einzustellende *on-line* Algorithmen für die Schätzung von Reaktionskinetiken entwickelt. Die Einstellung der Algorithmen ist von entscheidendem Einfluß auf die Entwicklungskosten. Die Untersuchungen charakterisieren die Beziehung zwischen Einstellung, Stabilität und Dynamik der Konvergenz. Das Verhalten der Algorithmen ist bereits vor ihrem ersten Einsatz bekannt. In Folge dessen besteht keine Notwendigkeit, die im allgemeinen hohe Kosten beinhaltende Versuch- und Fehler-Techniken durchzuführen.

Contents

Chapter 1

Introduction

Abstract. Process optimization, supervision and control are becoming an increasingly important issues due to hard competition between companies. Still, the acceptance of model-based methodologies remains rather limited in the industry, mainly because the benefit/cost ratio is not clearly attractive for such developments. In the classical model-based approach, mathematical models are used to describe the relevant bioprocess mechanisms. Mainly due to the intrinsic complexity of biological systems, bioprocesses are not yet well understood from a mechanistic point of view. The main implication to be noticed is that the synthesis of mechanistic mathematical models is governed by a rather restrictive compromise between simplicity and development costs. Unfortunately the common situation in the industry is that models developed at tolerable costs are not accurate enough to solve the underlying optimization, supervision and control problems. The main objective of the present Ph.D. dissertation is to give noticeable step ahead for increasing the benefit/cost-ratio of a model-based way of process improvement. With this respect two main research lines are defined: (i) Hybrid modeling as an efficient alternative to the classical mathematical modeling approach and (ii) efficient and easy-to-implement on-line adaptation algorithms.

1 MODEL-BASED SUPERVISION, CONTROL AND OPTIMIZATION OF BIOPROCESSES

As biotechnology becomes more developed and extended all over the world, the competition between different companies is becoming harder. The general consequence to be noticed is that the companies are taking a closer look to economics of their production processes.

From the biochemical engineering point of view, the straightforward way of improving the economics is to invest in process optimization and control.

In industrial practice, improvements are often achieved by more or less educated trial and error methods, i.e. by empirical methods guided by intuition and experience. Good luck is an essential condition to be successful in this way.

Others prefer systematic approaches, which, on the average, lead to a faster rate of improvements. The systematic approach is characterized by a consequent utilization of the *a priori* knowledge about the process. Usually, the number of experiments required can be kept much smaller in this way. Thus, we expect a significant reduction of expenses.

Most often, mathematical process models are considered to represent the *a priori* knowledge. When we speak about models in biochemical engineering and in particular with respect to process optimization and control, we think of relationships, which describe the basic aspects of the real process that are most important to process performance. One main demand is that the process models allow to represent the relevant process properties quantitatively and so make them accessible to efficient computational analysis and optimization techniques. In biotechnology, classical mathematical models are constructed using some mechanistic concepts, which are embedded into mass balances, i.e. a relatively simple set of differential equations (e.g. Sonnleitner and Käppeli, 1986). Recently, some alternative methods of representing bioprocesses were discussed in literature, in particular artificial neural networks and fuzzy expert systems (Thomson and Kramer, 1994, Lübbert and Simutis, 1994) are used to extend the classical approaches.

In order to solve the task of improving the process performance, it is indispensable to define beforehand how to measure the progress. What we need is a quantitative criterion formulated by means of an objective function. Such an objective function may be a rather complicated function, since it should not only consider the main objective, e.g. the optimization of the volumetric productivity with respect to the main product, but also the practical boundary conditions which must be met during production.

Once we have such an objective function for the process performance, we are also able to define what we are meaning by model performance: The performance of a model is simply the advantage it brings in improving the process performance with respect to the objective function.

Evidently, the number of industrial production reactors, where model supported process supervision, optimization, and control is applied, is very low. There are a number of reasons. The most significant ones arise in connection with the problem of activating enough knowledge about the process under consideration. Moreover, too complicated modeling and optimization procedures led to high development times. Therefore, in most practical attempts made so far the benefit/cost ratio appeared to be too low. Essentially both factors, the cost as well as the benefit, did not justify model supported optimization and control (Royce, 1993).

2 PROCESS MODELS FOR WHAT?

Models can be used to support bioprocess improvement in many different ways. In the present work the interest is devoted to those aspects related to process optimization, control and supervision. Three specific applications of models are treated in the present Ph.D. thesis. They are briefly introduced in the following lines.

A very important application of models is supporting the determination of optimal process trajectories. This is commonly referred to as process optimization or open-loop control (Simutis *et al.*, 1997). The main idea is the identification of optimal profiles for the relevant manipulated variables, such as substrate input feed rate, with the objective of imposing an optimal path to the process, according to a pre-established optimal process performance criterion. This optimal criterion must be carefully stated in the form of an objective function which must consider the relevant process constraints. Open-loop control requires accurate models for reproducing as close as possible the real response of the process to hypothetical profiles of the manipulated variables. A critical issue in this respect is related to the extrapolation capabilities of the model. Such a development is performed off-line relying completely on the accuracy and extrapolation capabilities of the process model. The important consequence to be noticed is that models for open-loop control must describe the relevant mechanisms related to the pre-established objective function.

Process dynamical models are often used to design control systems, what is commonly referred to as model-based control system design. In this respect models can be used to derive control algorithms and to tune the controller parameters involved. Properties like stability, robustness, and tracking dynamics of control variables to their setpoints must be carefully studied before the control system is tested in the real plant. When the underlying dynamical model is nearly time-invariant, and provided that there is enough process data to identify it within the process working region, the control system design can be done in an off-line fashion. Unfortunately the common situation is that biochemical processes have strong time-varying dynamics. To cope with the impossibility of developing models (and controllers based on these models) describing fully the dynamics of the process, on-line adaptation schemes must be implemented. Such a control strategy is usually termed as model-based adaptive control.

Another important application of models is developing software sensors. Software sensors enable the estimation of variables on-line which cannot be measured directly. The knowledge of these variables might be important to support other on-line applications such as monitoring, control, and fault detection. Quite often software

sensors are state estimators which enable the calculation of unknown variables from other variables easily accessible on-line. Software sensors can also be parameter estimators. A typical application is monitoring reaction kinetics. Software sensors require on-line process data and can also be adaptive.

3 THE CLASSICAL WAY OF DEVELOPING BIOPROCESS MODELS

In the classical approach of developing bioprocess models, mathematical relations are employed for describing the relevant mechanisms. From the engineering point of view, two main systems must be analyzed: (i) the bioreactor system and (ii) the biological cell system. Bioreactor models deal with mass transfer aspects and flow patterns in both gas and liquid phases. Cell models deal with the kinetics on the individual cell level and on the whole cell population level. The bioreactor system and the cell system have very complex interactions and cannot be analyzed separately. The living microorganisms are transforming continuously the liquid phase by consuming several nutrients, which are metabolized into several products, some of them excreted in the surrounding media. The metabolism is also influenced by several physical variables, such as temperature, pressure, light intensity, etc. In the present chapter, the discussion concerns a particular type of bioreactors, the stirred tank bioreactor, which is the one most often found in industrial production processes.

3.1 Transport processes

When developing a model for a bioreactor we are mainly interested on a description of the dynamics of those macroscopic quantities that influence the behavior of the microorganisms. They are concentrations in the broth of several components such as biomass, substrates, and products, and also several physical quantities such as temperature and pressure. These variables are usually called state variables since they define the process state. A mathematical dynamical description of the state variables is obtained by applying general mass, energy and momentum conservation laws (Roels *et al.*, 1978). The choice of the set of state variables to consider is very much dependent on the objective of the model. When, for instance, the temperature is kept constant by means of a very simple PID control, there is no need to include the variable temperature in the state space vector. This prevents the necessity of employing macroscopic energy balances for describing the process state. They make however sense if the objective of the model is designing the temperature PID controller, and specially in large scale bioreactors where cooling problems are likely to be found (Humphrey, 1998).

A very problematic issue concerns the rheological effects in bioreactors. In general the modeling of flow patterns is far too complex. And specially in the case of stirred tank bioreactors. The essential implication to be noticed is that in both industrial and research applications these effects are systematically ignored (Kleinstreuer, 1987). Almost invariably the assumption is taken that both liquid and gas phases are well mixed, i.e. the medium is assumed to be homogeneous. From the mathematical and numerical point of view this introduces an enormous simplification since, otherwise, an extremely complex analysis based on distributed parameter systems would have to be performed (e.g. Royce, 1996). Under this assumption, and by considering that temperature, pressure and pH are usually controlled quantities being kept at constant

values, there is solely the necessity of applying mass balance principles to the relevant components. This leads to the following set of ordinary differential equations:

$$\frac{dX}{dt} = R + \frac{F}{V}(X_{in} - X) + Q \qquad (1)$$

where X represents a vector of concentrations in the broth (the state space vector), R is a vector of reaction kinetics, F is the input feed rate into the bioreactor, V is the broth volume, X_{in} is a vector of concentrations in the input feed rate F, and Q a vector of gaseous outflow rates (such as oxygen and carbon dioxide transfer rates).

3.2 Modeling mass transfer between gas-liquid phases

With exception of cooling limitation problems usually found in large bioreactors (Kleinstreuer, 1987), the most frequently encountered limitation to growth in aerobic fermentations is dissolved oxygen in the broth (Thornhill and Royce, 1991). Oxygen has a very low solubility in fermentation media in comparison to other typical substrates (Stanbury and Whitaker, 1984). Therefore it must be continuously supplied, usually by aeration. The mass transfer capacity between gas and liquid phases is a central problem in aerobic bioprocesses design and operation. The mathematical description of the oxygen transfer rate from the gas phase into the liquid phase is based on general concepts of mass transfer theory. The main resistance for oxygen transfer from the gaseous phase into the medium lies in the liquid-side boundary layer of the gas-liquid interface. In general the following mass transfer law applies:

$$OTR = k_L a \, (O^* - O) \qquad (2)$$

where OTR is the oxygen transfer rate from the gaseous phase into the liquid phase, O is the dissolved concentration in the broth, O^* is the oxygen saturation concentration in the broth, and $k_L a$ the global mass transfer coefficient.

The application of law (2) poses two main difficulties, namely the knowledge of $k_L a$ and the knowledge of O^*. The solubility of oxygen in the broth is a function of the media composition, temperature and pressure. The dependency with temperature and pressure can be quantified accurately enough by applying Henry's law. However, the dependency with the medium composition is rather difficult to describe and is normally neglected (Pirt, 1975).

The global mass transfer coefficient is a very complex function of the bioreactor geometry, the impeller geometry, medium rheology, the aeration flow, the stirrer speed, and the composition of media where, for instance, viscosity is an important parameter. The mathematical description of $k_L a$ is usually made by means of empirical correlations, such as (Humphrey, 1998):

$$k_L a = K \left(\frac{P_g}{vol}\right)^a (V_s)^b (\mu)^c \qquad (3)$$

where (P_g/vol) is the gassed power per unit volume, (V_s) the superficial gas velocity and (μ) the broth viscosity. From the practical point of view, a very important issue is that the knowledge of the maximum $k_L a$ defines the most important constraint in bioprocess optimization. The correlation between $k_L a$, stirrer speed and air flow is also important for designing control systems for dissolved oxygen in the broth.

Carbon dioxide is a byproduct of cells metabolism. In many production processes, such as antibiotic production processes, the dissolved CO_2 inhibits the metabolism. Thus it must be stripped off the media. This is done with the same aeration equipment used to supply oxygen. In the case of CO_2 the mass transfer occurs in the opposite direction, i.e. from the liquid phase into the gaseous phase. The general mass transfer law applies:

$$CTR = k_L a\,(C^* - C) \tag{4}$$

where CTR is the carbon dioxide transfer rate from the liquid phase into the gaseous phase, C is the concentration of dissolved carbon dioxide in the broth, C^* is the dissolved CO_2 concentration directly at the physical interfacial area, and $k_L a$ the global mass transfer coefficient.

Under the assumptions of small-scale bioreactors and perfectly mixed gas and liquid phases, the values of $k_L a$ for CO_2 and O_2 transfer are related according to the following relationship (Thornhill and Royce, 1991):

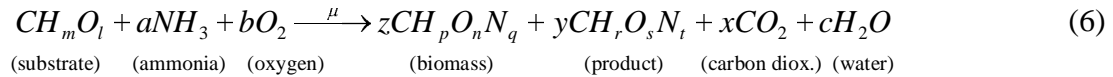$$(k_L a)_{CO_2} = (k_L a)_{O_2}\sqrt{\frac{D_{CO_2}}{D_{O_2}}} \tag{5}$$

where D_i refers to the diffusivities of oxygen and carbon dioxide in the liquid phase.

3.3 Modeling microorganism kinetics

The reaction term R in eqn. (1) is the result of an extremely complex metabolic reactions network on the cell level and on the whole cell population level. A mathematical description of the cell metabolism is far too complex and not of real practical use for industrial applications. In many aspects such an analysis is impossible because many metabolic mechanisms are still unknown or cannot be validated with experimental measurements. The common situation is that mathematical descriptions of microorganism kinetics are rough simplifications of the reality.

Several kinds of cell models have been proposed in the literature. In general they can be classified in structured/unstructured and segregated/non-segregated (Tsuchiya *et al.*, 1966). Cell models considering the existence of intracellular components are termed structured, otherwise they are termed unstructured. They may also assume the existence of a morphological structure, being then termed segregated models, or may assume that all cells are identical (only one morphological form) being then termed non-segregated models.

For bioprocess optimization, control and supervision, unstructured and non-segregated models are the only ones of practical interest. In developing such models, principles of macroscopic stoichiometry and empirical kinetic correlations are employed. The starting point of such an analysis is the establishment of a set of biochemical equations describing the most relevant mechanisms in the cell. In the most simple version of such an analysis, only a single biochemical reaction is considered, describing the whole cell metabolism. For instance, a biochemical aerobic reaction where one substrate, ammonia and oxygen are consumed, producing biomass, one product, carbon dioxide and water, with a conversion rate of μ, is stated in the following way:

$$CH_mO_l + aNH_3 + bO_2 \xrightarrow{\mu} zCH_pO_nN_q + yCH_rO_sN_t + xCO_2 + cH_2O \qquad (6)$$

(substrate)　(ammonia)　(oxygen)　　　(biomass)　　　(product)　(carbon diox.) (water)

When the elemental composition of all the species involved is known it is possible to evaluate the molar stoichimetric coefficients a, b, y z x and y by applying general mass and energy balance principles (Roels, 1978; Minkevich and Eroshin, 1975). The well-known yield coefficients in biotechnology are related to these stoichiometric coefficients by simple molar- to mass-base transformations. For instance, the yield coefficient of biomass production per 1 gram of substrate consumption is given by the following equation:

$$Y_{x/s} = z\,M_x/M_s \qquad (7)$$

where M_x and M_s are the molecular weights of biomass and substrate respectively.

Once the yield coefficients are known, a further model for the specific growth rate μ is necessary. The consumption and production kinetics of all the species are linked together by the stoichiometry, and the specific growth rate μ. For instance the consumption rate of substrate is given by:

$$R_s = \mu X/Y_{x/s} \qquad (8)$$

The mathematical descriptions of specific growth rates are in general based on semi-empirical correlations. For the most industrial applications the following four models are employed:

1. The Monod model (Monod, 1942). This model considers substrate limitation at low concentrations:

$$\mu = \frac{\mu_{max}\,S}{K_S + S} \qquad (9)$$

2. The Moser model (Moser, 1958). This is an extension of the Monod model.

$$\mu = \frac{\mu_{max}\,S^N}{K_S + S^N} \qquad (10)$$

3. The Haldane model (Haldane, 1942). This model considers substrate limitation at low concentrations and substrate inhibition at high concentrations

$$\mu = \frac{\mu_{max}\, S}{(K_S + S)(S + K_i/S)} \tag{11}$$

4. The Contois model (Contois, 1959). The Contois model is an extension of the Monod model, considering an inhibitory effect of cells concentration on growth (useful in high cell density cultivations).

$$\mu = \frac{\mu_{max}\, S}{K_S X + S} \tag{12}$$

The use of the above mentioned kinetic models requires a previous identification of the parameters involved. For instance, when using the Monod model (9) the maximum specific growth rate μ_{max}, and the saturation coefficient K_s must be identified for the actual cultivation conditions. Unfortunately even for such a simple kinetic relationship as the Monod model, this identification is rather difficult, requiring a careful experimental planing (Baltes *et al.*, 1994; Munack, 1989). In general the coupling of Monod-type kinetic models with mass balance equations forms non-linear dynamical systems, which, depending on the its structure, may not be identifiable. In the cases when they are identifiable theoretically, a careful (and expensive) experimental design is required.

4 THE HYBRID MODELING APPROACH

Hybrid modeling is emerging in the last years as a valid alternative to the classical modeling approach (Schubert *et al.*, 1994a; Psichogios and Ungar, 1992; Thomson and Kramer, 1994; Feyo de Azevedo *et al.*, 1997). It is further a very attractive methodology for applications to biochemical processes due to its intrinsic complexity. In the present section, the different kinds of knowledge usually available for biotechnological processes are characterized. The modeling methods suitable for efficient representation of each kind of knowledge are briefly discussed. Finally, some typical hybrid model structures usually found in the literature are overviewed.

4.1 Knowledge and representation of knowledge

Normally there is a variety of information sources on biotechnological cultivation processes (Schubert *et al.*, 1994a; Lübbert and Simutis, 1994). There are 3 main types of knowledge available, namely (i) the mechanistic (phenomenological) knowledge, (ii) heuristic knowledge and common sense, and (iii) knowledge hidden in the process data records. These sources of knowledge can be classified according to its level of sophistication and resolution of details (see Fig. 1).

4.1.1. The mechanistic (phenomenological) knowledge. This kind of knowledge is usually represented by mathematical models. This is the classical approach followed by chemical and biochemical engineers for developing their process models (e.g., Volesky and Votruba, 1992; Nielsen and Villadsen, 1994; Roels, 1983). It has the highest level

of sophistication, involving the understanding of the basic transport and kinetics mechanisms. These mechanisms are often poorly understood or even completely unknown. Therefore this kind of knowledge is usually the one available in minor quantities.

4.1.2. Heuristic knowledge and common sense. This kind of knowledge is more qualitative then the former, but it is usually available in major quantities in the industrial environment. The Fuzzy theory, first developed by Zadeh (1973), is often used to build heuristic knowledge based models. This theory provides methods for qualitative knowledge representation with mathematical precision. Heuristic knowledge is often stated in terms of rules of thumb. These can be readily represented by the so-called knowledge based systems such as fuzzy inference systems and expert systems (e.g. Sugeno, 1985; Kosko, 1992; Wang, 1994).
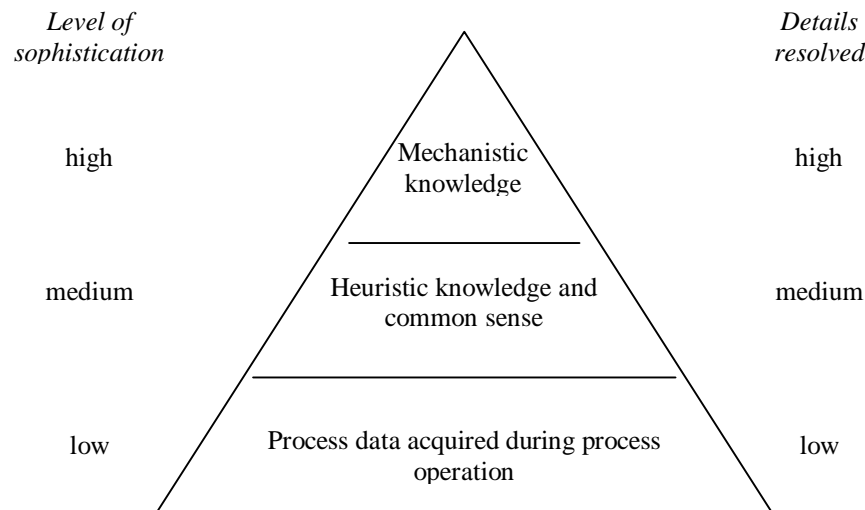
<table>
<tr><td>*Level of sophistication*</td><td></td><td>*Details resolved*</td></tr>
<tr><td>high</td><td>Mechanistic knowledge</td><td>high</td></tr>
<tr><td>medium</td><td>Heuristic knowledge and common sense</td><td>medium</td></tr>
<tr><td>low</td><td>Process data acquired during process operation</td><td>low</td></tr>
</table>

Fig 1. Hierarchical structure of process knowledge, level of sophistication of its components and resolution of details

4.1.3. Knowledge hidden in the process data acquired during process operation. Unfortunately, in many situations the available mechanistic and/or heuristic is not sufficient to develop a process model with the desirable accuracy. In this situation data-driven modeling methods can be used to improve the accuracy of the model. In many industrial plants the relevant cause/effect mechanisms have been registered for decades in the form of process input/output data. Some of these mechanisms have been at least observed by the people operating the plant, but many of them have been just recorded in process data files and passed completely unaware. The modeling of unknown parts of the process can be made using the so-called black box methods such as splines, polynoms, fourier series, or artificial neural networks (ANN). A very complete survey of black-box modeling in system identification is given by Sjöberg *et al.* (1995). In particular ANNs have been getting a great deal of attention from researchers in the last years. They proved to be extremely flexible in representing complex non-linear relationships (e.g., Cybenko, 1989; Hornik *et al.*, 1989; Poggio and Girosi, 1990)

without requiring any kind of knowledge concerning the structure of the underlying model. Several important results have been published concerning the application of ANNs for dynamical system identification and control (e.g., Hunt *et. al.*, 1992; Pollard *et al.*, 1992; Narendra and Parthasarathy, 1990). ANNs are of particular interest for bioprocess modeling due to the intrinsic complexity of bioprocesses.

4.2 Efficient knowledge fusion

Normally the different kinds of knowledge about a process are complementary but in many situations they overlap. This essentially means that some parts of the process can be simultaneously represented at different levels of sophistication. Different representations can perform better in some regions of the input space and worst in some other regions. In this sense the different kinds of knowledge are complementing themselves. As such, in hybrid modeling methods for knowledge weighting are of central importance. These weighting methods have the task of deciding which models perform better for the current set of model inputs, and according to this, to dynamically weight the outputs of each different representation for the evaluation of the final model outputs (see Fig. 2). These weighting methods are responsible for efficient knowledge fusion.
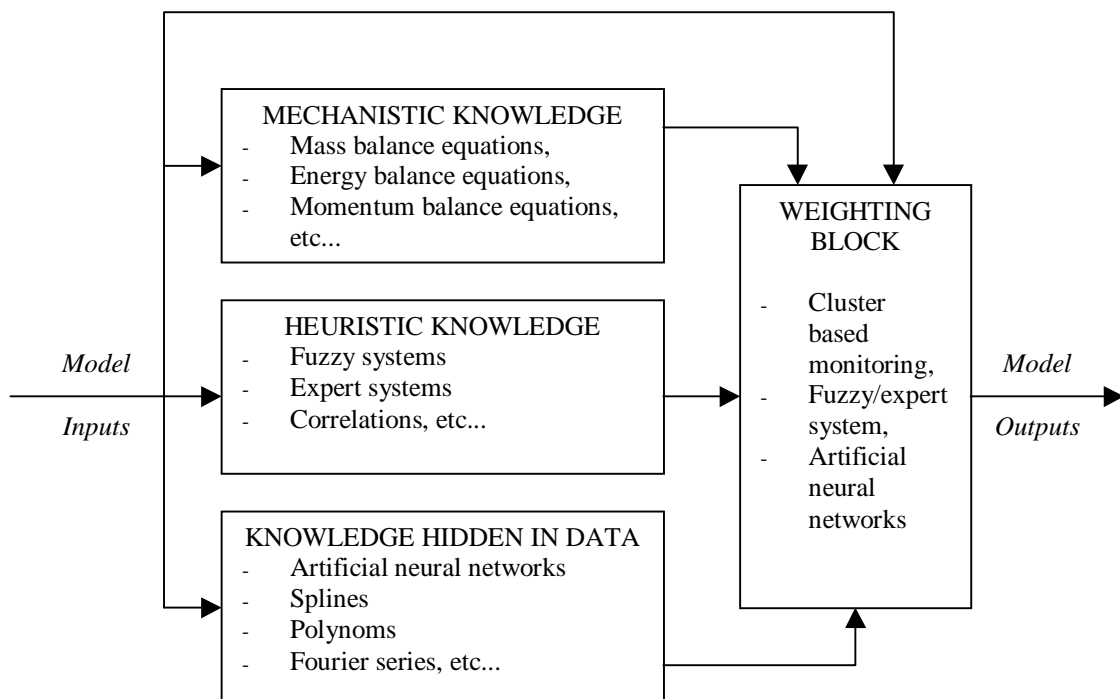

Fig. 2. Generic way of incorporating different kinds of *a priori* information in a single model structure: physical knowledge, heuristic knowledge and knowledge hidden in process data. The weighting block must evaluate dynamically the relative weight of different kinds of representations for the final evaluation of the model output.

Some examples of weighting methods have been reported in the literature, from which the most important are:

1) Weighting methods based on clustering techniques (e.g., Simutis *et al.*, 1995; Leonard *et al.*, 1992)
2) Weighting methods based on expert systems (e.g., Schubert *et al.*, 1994b)
3) Weighting methods based on artificial neural networks (e.g., Haykin, 1994)

Clustering techniques can been used for monitoring the reliability of artificial neural networks (e.g., Simutis *et al.*, 1995; Leonard *et al.*, 1992). The main idea is to represent in a compact way the input space (see Fig. 3) which defines the domain of experience of the artificial neural network being monitored. In this way it is possible to evaluate the extrapolation measure of the neural network prediction for given input vectors. This extrapolation measure is the base of decision on using the neural network model or some other model with better extrapolation properties.
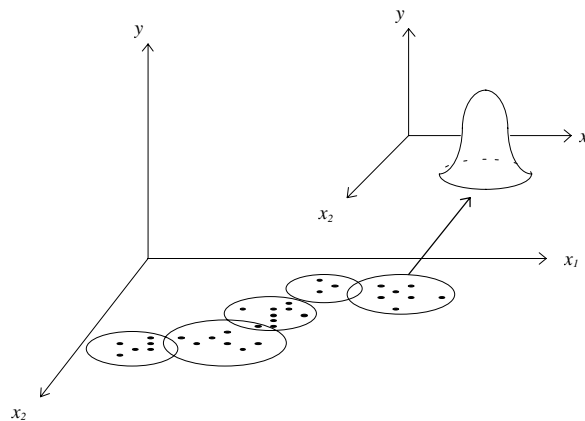
Fig. 3. Clustering of a 2-dimensional input data space by means of multivariate gaussian shaped functions

Another typical approach is based on Fuzzy systems. It can be applied when there is sufficient heuristic knowledge about the models' performance in different parts of the input space. A typical set of rules is as follows:

RULE 1. *IF*   *input space is completely unknow* *THEN*   *use only mechanistic model*
RULE 2. *IF*   *input space is poorly known*   *THEN*   *use mechanistic and heuristic models*
          ⋮   ⋮
RULE N. *IF*   *input space is completely known*   *THEN*   *use only ANN model*

This approach is again based on the interpolation and extrapolation capabilities of different kinds of modeling techniques. An example of such a Fuzzy weighting method is given by Schubert *et al.* (1994b).

4.3 Typical hybrid model structures

Several hybrid model structures have been published in the literature. An important class of these structures are the neural-fuzzy systems. The neural-fuzzy systems combine artificial neural networks and fuzzy logic in one single model structure(e.g.,

Gupta and Rao, 1994; Werbos, 1992; Shi and Shimizu, 1992; Lin and George Lee, 1991).

Another important approach for designing hybrid model structures is based on the division of the process in study in several modules according to the kind of knowledge available in different parts of the process. The result of such an operation is usually expressed by a diagram of interconnected modules. Each module is expressed by an input-output relationship based on a particular modeling technique.

Several authors have been using a very simple hybrid model structure (e.g., Wilson and Zorzetto, 1997; Psichogios and Ungar, 1992; Montague and Morris, 1994; Feyo de Azevedo *et al.*, 1997) based on the use of artificial neural networks for describing the microorganism kinetics embedded into mass balance equations (see Fig. 4). However, this structure has major a drawback related to the poor extrapolation capabilities of neural networks. Such a model can be only used within the data space used before for training the underlying artificial neural network.
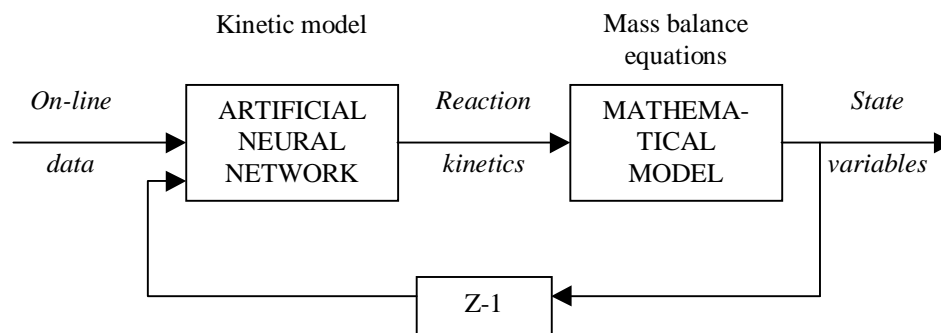
Fig 4. Popular hybrid model structure based on an artificial neural networks for describing the microorganism kinetics embedded into a set of mass balance equations.

To improve the general extrapolation capabilities of such a hybrid model structure Simutis et al (1996) suggested to include a safety model (see Fig. 5) which should be used whenever the ANN is operating in extrapolation conditions. In this way we are improving the global extrapolation properties of the model. Most often the safety model is based on Monod relationships or on Fuzzy models. The latter can only be employed when there is enough heuristic knowledge in the form of *IF(...) THEN (...)* rules for describing the microorganism kinetics.
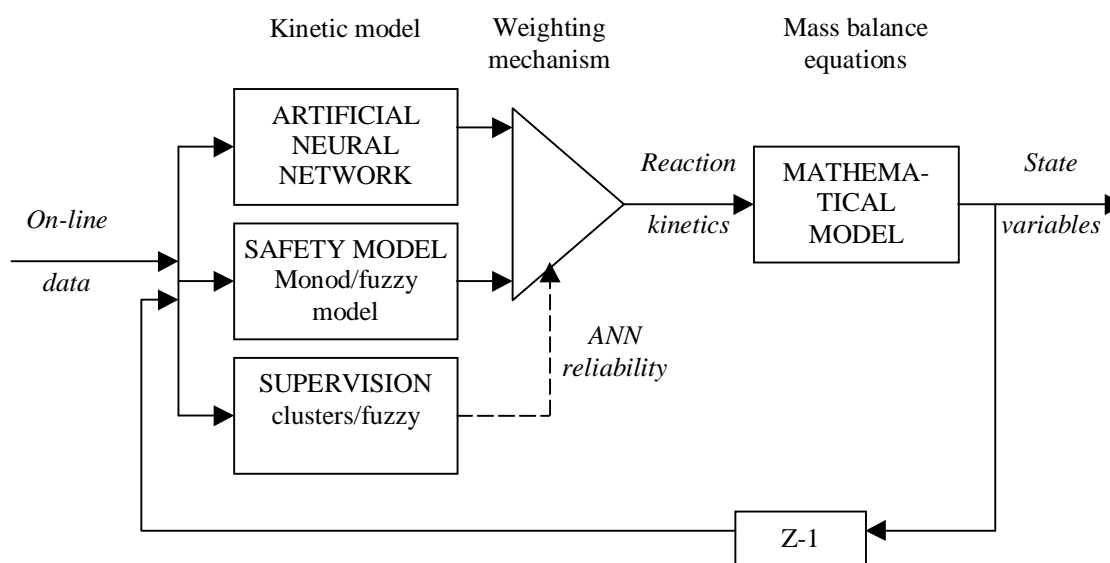
Fig 5. Typical hybrid model structure based on the combination of a kinetic model with mass balance equations. The kinetic model is based on two complementary representations: an ANN and a safety model. The ANN and the safety model outputs are dynamically weighted according to a mechanism ruled by a supervision block which is continuously detecting whether the ANN is extrapolating or not. Whenever the ANN is extrapolating, its relative weight is decreased while the one of the safety model is increased.

5. AIMS AND SCOPE OF THE THESIS

The benefit/cost-ratio is the quantity that rules the acceptance of model-based process supervision, optimization and control in industrial scale chemical and biochemical production processes. Unfortunately this ratio has been too low for the most applications. The central problem discussed in the present Ph.D. thesis is the following:

Problem: How is it possible to increase the benefit/cost-ratio of a "model-based" way of biochemical process improvement?

Nowadays it is openly accepted that biochemical processes are very complex and often poorly understood. This is the main reason why in general it is very difficult to develop a mechanistic model with the required accuracy. The synthesis of a process model in the classical way is slow and expensive because it is based on a progressive comprehension of the phenomena involved. The bottleneck here is the acquisition of knowledge. Clearly, the development of bioprocess models will always be expensive. But how much can we reduce these expenses?

Problem: How is it possible to reduce the costs of developing a biochemical process model for given accuracy requirements?

Since the accuracy of a process model is a function of the quantity and quality of the knowledge available, a very important issue to explore is the rational and efficient use

of knowledge for model synthesis. This represents a very important issue in the present Ph.D. thesis, namely hybrid modeling and its applications to biochemical processes. Hybrid modeling provides means of activating all the sources of knowledge available, hence it is a way of getting more accurate process description at less costs (hence promising to enhance the benefit/cost-ratio for industrial applications)

Aim 1. To develop the concept of hybrid modeling and its applications to bioprocess model based improvement

In those situations when on-line process information is available, model-based algorithms can be complemented with on-line adaptation algorithms. In this case, and in relation to the discussion about the benefits and costs of a model-based process improvement, a compromising solution between:

1) complexity/simplicity of process model
2) complexity/simplicity of on-line adaptation algorithm.

can be taken. The second aim of the present Ph.D. thesis is to develop algorithms based on very simple process models complemented with on-line adaptation algorithms.

Aim 2. To develop algorithms based on very simple process models complemented with on-line adaptation algorithms

Since the optimal utilization of on-line process information is of crucial importance, it is important to explore the possibility of using hybrid models completed with on-line adaptation strategies. Since this issue is not yet well developed, the third aim of this Ph.D. thesis is:

Aim 3. To develop on-line adaptation strategies for complementing algorithms based on hybrid models

The last aim which is also of crucial importance, concerns the practical implementation of these methodologies in the industrial environment. The practical implementation of hybrid models requires appropriate software tools not yet available in the market. So the last aim of the present Ph.D. is:

Aim 4. To develop a complete software package to support the implementation of hybrid model-based algorithms for process optimization, control and supervision. The software must be flexible and user-friendly so that it can be accepted in a typical industrial environment.

6. OUTLINE OF THE THESIS

The present Ph.D. thesis is organized in 7 chapters. In chapter 1 (the present chapter) the motivations, aims and scope of the present dissertation are discussed. Some relevant introductory concepts for the developments in the following chapters are also presented. First of all the motivation is clarified, i.e. why is it important to perform biochemical process improvement, and why is the acceptance of the model-based approach so poor

in the industrial practice. It is explained that it is very important to reduce the costs of modeling, and with this respect some research lines were established. This resulted in a set of particular issues that are going to be subject to careful study.

Chapters 2, 3 and 4 address the problem of hybrid modeling and its applications to biochemical processes improvement. In chapter 2 a general framework for hybrid modeling is developed based on hybrid network model structures. The approach is illustrated by the application to state estimation and open-loop in a fed-batch baker's yeast cultivation process. In chapter 3 the HYBNET software package, developed in the ambit of the present Ph.D. dissertation, is presented. The software concept is illustrated by the application to open-loop and closed-loop optimization of a fed-batch baker's yeast cultivation process. Chapter 4 describes an application of hybrid-model networks and of the HYBNET software package for designing and implementing a closed-loop inferential control system in a penicillin production process.

Chapter 5 and 6 address the problem of estimating process state and process parameters on-line and in real-time. These two chapters focus on methodologies that use on-line information to increase robustness. They exemplify how it is possible to use on-line process data rationally to cope with model inaccuracies. This is further one of the fundamental questions in the model-based way of process improvement: should more resources be invested into model development or into robust schemes for on-line adaptation to cope with the impossibility of the model to describe fully the dynamics of the process? The development of accurate kinetic models is a critical issue in bioprocess modeling. These 2 chapters cover extensively the subject of reaction kinetics estimation from variables usually available on-line. These algorithms are examples of methods that avoid to rely on models of parts of the process that are poorly understood. The methods are illustrated using simulation experiments.

In chapter 7 the general conclusions to be taken from the present dissertation are presented. Concern is given to explain in which way the preliminary defined aims in the introduction chapter were achieved.

Chapters 2 to 6 are based on 5 publications. They are briefly summarized in the following lines.

Chapter 2. Hybrid Networks: A New Approach for Bioprocess Modeling. A new approach to system identification for process supervision and control using hybrid modeling techniques is presented and illustrated with implementations at baker's yeast fed-batch cultivation process. The conceptual basis of the new method is the arrangement of the modules of the hybrid model in the form of a network. Thus, a hybrid network is a computational structure consisting of a network of computational nodes, which represent process knowledge at different levels of sophistication. Two main aspects of hybrid network modeling are addressed: (i) construction of hybrid networks by combining different a priori information or knowledge about the process under consideration, and (ii) efficient parameter identification for hybrid networks. The most important property of hybrid networks is that the error backpropagation technique can be applied in order to significantly simplify variable sensitivity analysis and parameter identification. The backpropagation technique was extended to dynamic

systems. Two applications serve to demonstrate the advantage of the concept of hybrid networks: (i) biomass estimation, (ii) open-loop optimization.

Chapter 3. HYBNET, an Advanced Tool for Process Optimization and Control. This paper describes the software package HYBNET developed during the present Ph.D. work. HYBNET is a software package that supports advanced model-based process design and operation. HYBNET stands for HYBrid NETwork, since it is able to compose the process model in a modular way and allows to connect these nodes in form of a network. It is hybrid in different respects: First, the models representing the different parts of the process or its behaviour in different situations can be formulated at different levels of sophistication, corresponding to the knowledge available about that particular aspect of the process. In most applications the modules are formulated in different representations like neural networks, fuzzy rule-based models and classical differential equations. More than a hundred of such different nodes can be interconnected in a most flexible way in order to form a well performing process model as the base for systematic process optimization, process supervision and closed-loop control. The nearly arbitrarily structured models can be identified in roughly the same way as the training of artificial neural networks, namely by means of error backpropagation schemes. One of the most essential advantages from the practical point of view is that the software package is nearly platform independent. It only requires a link, e.g. with a TCP/IP protocol, to the process control computer used. The concept of the software is illustrated at the example of a baker's yeast production process.

Chapter 4. Closed-loop Control Using an On-line Learning Hybrid Model Network. This chapter presents a model-based closed-loop control procedure based on a hybrid process model. An inferential control strategy not yet discussed in literature was chosen to keep the concentrations of ammonia and precursor in penicillin production experiments under tight control. Its main component, the estimator for the ammonia and precursor consumption rates, is an indirect measurement procedure, which uses several different on- and off-line measurement data. An hybrid process model was taken to combine several kinetic models, which was capable of learning during its application using automatic training techniques. Particularly, the neural network component of the hybrid network was retrained during this on-line learning process. Also, all the other components of the hybrid model are automatically re-tuned, once new data becomes available. The proposed procedure was tested in 22 fermentation runs where it proved to be robust and stable. The method exemplifies how is it possible to use hybrid model based algorithms together with on-line adaptation.

Chapter 5. A Study on the Convergence of Observer-based Kinetics Estimators in Stirred Tank Reactors. In this paper a model-based parameter estimator is proposed for the on-line estimation of reaction rates in stirred tank bioreactores. A particular attention is given to the stability requisites and the dynamics of convergence of the estimates to the true values. These two fundamental issues are discussed in relation to the tuning procedure of the gain parameters. The application of the algorithm is illustrated with a simple microbial growth cultivation process.

Chapter 6. On-line State Observation and Reaction Rates Estimation in a Baker's Yeast Cultivation Process. In this paper algorithms for state observation and kinetics

estimation are developed and applied to a baker's yeast fed-batch cultivation process. An important design condition was to keep the number of required on-line measurements as low as possible. The overall estimation scheme aims at the estimation of three state variables and three specific growth rates requiring on-line measurements of dissolved oxygen, dissolved carbon dioxide and off-gas analysis. A great deal of attention is given to the estimation problem of reaction kinetics. In this respect a new algorithm is proposed -the Second Order Dynamics Estimator (SODE)- and compared to an Observer Based Estimator (OBE). Stability and dynamics of convergence are issues subject to detailed analysis. The numerical implementation related to the overcoming stability problems is also studied. It is shown that a discrete-time formulation poses additional stability constrains. These can be easily overcome by using a robust variable step integration algorithm. It was concluded that the OBE has two main disadvantages: i) the tuning of the design parameters must be done on a trial-and-error basis, while in the SDOE the user can set a 2nd order dynamics of convergence from estimated kinetics to "true" kinetics, and ii) the dynamics of convergence of the OBE are time-varying while in the case of the SDOE this response is time-invariant.

REFERENCES

Bailey, J.E., D.F. Ollis (1986). Kinetics of substrate utilization, product formation and biomass production in cell cultures. In *Biochemical Engineering Fundamentals*, McGraw-Hill, New York, USA, pp. 658-752

Baltes, M., R. Schneider, C. Sturm, M. Reuss (1994). Optimal Experimental Design for Parameter Estimation in Unstructured Growth Models. *Biotechnol. Prog.*, **10**, pp. 480-488

Contois, D.E. (1959). Kinetics of bacterial growth: Relationship between population density and specific growth rate of continuous culture, *J. Gen. Microbiol.*, **21**, pp. 40

Cybenko, G. (1989) „Approximation by Superpositions of a Sigmoidal Function," *Math. Control, Signals Sys.,* **2**, pp. 303-314

Feyo de Azevedo, S., B. Dahm, F.R. Oliveira (1997) "Hybrid Modelling of Biochemical Processes: A comparison with the conventional approach," *Computers chem. Engn*, **21**, Suppl., pp. 751-756

Gupta, M.M. and D.H. Rao (1994). On the principles of fuzzy neural networks. *Fuzzy Sets and Systems*, **61**, pp. 1-18

Haykin, S. (1994). Neural Networks: a Comprehensive Foundatation. *IEEE Press*, New York

Hornik, A. K. and M. Stinchcombe, H. White (1989) „Multi-Layer Feedforward Networks are Universal Approximators," *Neural Networks,* **2(5)**, pp. 359-366

Humphrey, A. (1998). Shake Flask to Fermentor: What Have We Learned? *Biotechniol. Prog.*, **14**, pp. 3-7

Hunt, K.J., D. Sbarbaro, R. Zbikowski and P. J. Gawthrop (1992) „Neural Networks for Control Systems-A Survey," *Analitica*, **28(6)**, pp. 1083-1112

Kneinstreuer, C. (1987). Analysis of biological reactors. In *Advances in Biochemical Engineering*, H. Bungay and G. Belfont, eds., Wiley, New York, USA, pp. 33-78

Kosko, B. (1992). Neural networks and fuzzy systems: a dynamical systems approach to machine intelligence. Prentice-Hall, Englewood Cliffs, New Jersey

Leonard, J.A., M.A. Kramer, L.H. Ungar (1992). A neural network that computes its own reliability. *Comp. Chem. Eng.*, **16**, pp. 819-835

Lin, Chin-Teng and C.S. George Lee (1991).Neural-Network-Based Fuzzy Logic Control and Decision System. *IEEE Transactions on computers*, **40(12)**, pp. 1320-1336

Lübbert, A., R. Simutis (1994) "Adequate use of measuring data in bioprocess modeling and control," *Trends in Biotechnology*, **12**, pp. 304-311

Minkevich, I.G. and V.K. Eroshin (1975). *Stud. Biophys.*, **49**, pp.43

Monod, J. (1942). Reserches sur la croissance des cultures bacteriennes, Paris: Herrmann et Cie

Montague, G., J. Morris (1994). Neural network contribution in biotechnology. *Trends Biotechnol.*, **12**, pp. 312-324

Moser, A. (1958). The dynamics of bacterial populations maintained in the chemostat, Publication 614, Washington, DC: The Carnegie Institution

Munack, A. (1991) "Optimization of Sampling, In Biotechnology," Rehm and Reeds, Eds.; Verlag Chemie: Weinheim,; Vol.4: pp. 252-264.

Munack, A. (1989). A Optimal feeding strategy for identification of Monod-type models by fed batch experiments. In *Reprints of the 4th International Congress on Computer Applications in Fermentation Technology*, N.M. Fish, R.I. Fox, Eds., Ellis Horwood Limited: Chichester, U.K.

Narendra, K.S., and K. Parthasarathy (1990). Identification and control of dynamical systems using neural networks. *IEEE Trans. Neural Networks*, **1**, pp. 252-262

Nielsen, J., and J. Villadsen (1994). Bioreaction Engineering Principles. Plenum Press, New York

Pirt, S.J. (1975). Principles of Microbe and Cell Cultivation. Halsted Press, Wiley & Sons, New York, USA

Poggio, A. T. and F. Girosi (1990) „Networks for Approximation and Learning," *Proc. IEEE,* **78(9)**, pp. 1481

Pollard, J.F., M.R. Broussard, D.B. Garrison, K.Y. San (1992). Process identification using neural networks. *Comp. Chem Eng.*, **16**, pp. 253-270

Psichogios, D.C. and L.H. Ungar (1992). A hybrid neural network - First principles approach to process modelling. *AIChE J.*, **38**, pp. 1499-1511

Roels, J. (1983). Energetics and kinetics in biotechnology. Elsevier Biomedical Press, Amsterdam

Roels, J.A., N.W.F. Kossen (1978). On the modelling of microbial metabolism. *Prog. Ind. Microbiol.,* **14**, pp 45-58

Royce, M. (1996). Fermentation – the relation between intracellular metabolism and reactor dynamic. In Proceedings of the *1st European Symposium On Biochemical Engineering Science*, Dublin, Ireland, Septemper 19-21, pp. 3

Royce, P.N. (1993) "A discussion of recent developments in fermentation monitoring and control from a practical perspective," *Critical reviews in Biotechnology,* **13(2)**, pp. 117 -149.

Schubert, J., R. Simutis, M. Dors, I. Havlik, A. Lübbert (1994). Bioprocess optimization and control: Application of hybrid modelling. *Journal of Biotechnology*, **35**, pp. 51-68

Schubert, J., R. Simutis, M. Dors, I. Havlik, A. Lübbert (1994a). Hybrid Modelling of Yeast Production Processes – Combination of a priori knowledge on Different levels of Sophistication. *Chem Eng. Technol.*, **17**, pp. 10-20

Shi, Z. and K. Shimizu (1992). Neuro-fuzzy control of bioreactor systems with pattern recognition. *J. Ferment. Bioeng.*, 74, pp. 39-45

Simutis, R., I. Havlik, F. Schneider, M. Dors, A. Lübbert (1995). Artificial Neural Networks of Improved Reliability for Industrial Process Supervision. Preprints of the 6th Int. Conference on Computer Applications in Biotechnology, Garmisch-Partenkirchen, Germany, pp. 59-65, May 14-17, 1995

Simutis, R., R. Oliveira, M. Manikowski, S. Feyo de Azevedo, A. Lübbert (1997). How to increase the performance of models for process control and optimization. *J. Biotechnol.,* **59**, pp. 73-89

Sjöberg, J., Q. Zhang, L. Ljung, A. Benveniste, B. Delyon, P. Glorennec, H. Hjalmarsson, A. Juditsky (1995). Nonlinear Black-box Modeling in System Identification: a Unified Overview. *Automatica*, **12**, pp. 1691-1724

Sonnleitner, B., O. Käppeli (1986) "Growth of Saccharomyces cerevisiae is controlled by ist limited respiratory capacity: Formulation and verification of a hypothesis," *Biotechnol. Bioeng.*, **28**, pp. 927-937

Standbury, P., A. Whitaker (1984). Principles of Fermentation Technology. Pergamon Press

Sugeno, M. (1985). Industrial applications of fuzzy control. *North-Holland*, Amsterdam

Thomson, M.L., M.A. Kramer (1994) "Modeling chemical processes using prior knowledge and neural networks," *AIChE J.*, **40**, pp. 1328-1340.

Thomson, M.L., M.A. Kramer (1994). Modeling chemical processes using prior knowledge and neural networks. *AIChE J.*, **40**, pp. 1328 - 1340.

Thornhill, N.F., P.N.C. Royce (1991). Modelling Fermenters for Control. In *Measuring and Control in Bioprocessing*, K.G. Carr-Brion, ed., Elsevier, New York, USA, pp. 74-77

Villermaux, J. (1996) "Future Prospects for Chemical Engineering Research and Technology," *Chem. Tech. Europe*, January/February, pp. 21-23

Volesky, B. and J. Votruba (1992). Modeling and Optimization of fermentation processes. Elsevier, Amsterdam

Wang, H. Y., C.L. Cooney, D.I.C Wang (1977). Computer aided bakers' yeast fermentation. *Biotechnol. Bioengng*, **19**, pp. 69-86

Wang, Li-Xin (1994). Adaptive fuzzy systems and control: design and stability analysis. Prentice-Hall, Englewood Cliffs, New Jersey

Werbos, P.J. (1992). Neurocontrol and Fuzzy Logic: Connections and Designs. *International Journal of Approximate Reasoning*, **6(185)**, pp. 185-219

Westerterp, K., J. van Dierendonck, J. Kraa (1963). Interfacial areas in agitated gas-liquid contactors. *Chem. Engng Sci.*, 18, pp. 157-176

Wilson, J.A., L.F.M. Zorzetto (1997) "A generalised approach to process state estimation using hybrid artificial networks/mechanistic models," *Computers chem. Engng*, **21(9)**, pp. 951-963

Zadeh, L.A. (1973). Outline of a new approach to the analysis of complex systems and decision processes. *IEEE Trans. Sys. Man Cybern*, **3**, pp. 28-44

Tsuchiya, H.M., A.G. Fredrickson, R. Aris (1966). Dynamics of Microbial Cell Populations. *Adv. Chem. Eng.*, **6**, 125

Chapter 2

Hybrid Networks
A New Approach to Bioprocess Modeling

Abstract. A new approach to system identification for process supervision and control using hybrid modeling techniques is presented and illustrated with implementations at baker's yeast fed-batch cultivation processes. The conceptual basis of the new method is the arrangement of the modules of the hybrid model in the form of a network. Thus, a hybrid network is a computational structure consisting of a network of computational nodes, which represent process knowledge at different levels of sophistication. Two main aspects of hybrid network modeling are addressed: (i) construction of hybrid networks by combining different a priori information or knowledge about the process under consideration, and (ii) efficient parameter identification for hybrid networks. The most important property of hybrid networks is that the error backpropagation technique can be applied in order to significantly simplify variable sensitivity analysis and parameter identification. The backpropagation technique was extended to dynamic systems. Two applications serve to demonstrate the advantage of the concept of hybrid networks: (i) biomass estimation, (ii) open-loop optimization.

1 INTRODUCTION

Models are considered a vehicle for representing our current knowledge about the relevant properties under consideration in order to simplify the solution of the various tasks to be solved. Here we restrict ourselves to tasks in process supervision and control of biochemical production processes. In this domain, it was tried to base most models on physical insight and to formulate them by mathematical equations. As it is the amount of knowledge that can be activated what is limiting most practical control applications, it is necessary to make use of all information and knowledge available, irrespective of the level of sophistication by which it can be represented. In industrial practice, most knowledge is available in the form of heuristic rules gained from experience with various production processes, while crisp mechanistic descriptions in form of mathematical models are available only for some parts or aspects of the processes under consideration. Schubert *et al.* (1994a) showed for a very simple hybrid system the advantage of combining mechanistic knowledge with heuristic rules of thumb and information still hidden in data from experiments undertaken previously.

There are several approaches to hybrid modeling discussed in literature (Schubert *et al.*, 1994; Psichogios and Ungar, 1992; Thomson and Kramer, 1994; Feyo de Azevedo *et al.*, 1997). They all divide the process into subsystems and describe them by different kinds of representations. In biochemical cultivation processes, for example, it is most convenient to describe the macroscopic mass balances by an ordinary differential equation system and the biochemical conversion rates by means of an artificial neural network (Schubert *et al.*, 1994b). Other approaches combine heuristic rule systems, represented by fuzzy rules and processed using fuzzy logic with the information from extended data records which can be represented by artificial neural networks. Such neural-fuzzy systems (e.g., Gupta and Rao, 1994; Werbos, 1992; Shi and Shimizu, 1992; Lin and George Lee, 1991) can be considered a means of introducing heuristic *a priori* knowledge into the black-box models represented by artificial neural networks in order reduce the size of the data required to teach them behaving like the process under consideration. The different modules in hybrid models, however, need not necessarily describe different parts of the process, different modules might also describe the same part. Then, they can be taken as different votes to the values of the corresponding output variables of that process component, i.e. the modules are considered as alternative descriptions performed from different points of view. Then, however, a reliable weighting of the different results is required.

A high flexibility of composing the final process model from the various modules can obviously be obtained by arranging them in form of an extended network. When this is done in such a way that the flow of information from the modules containing the input variables of the process to the ones that provide the process' output values is similar to a feedforward neural network, then a very efficient parameter identification procedure becomes possible. Then it is possible to make use of the error backpropagation technique that allowed to simplify the tuning of the weights of artificial neural networks. Hence, the approach proposed by Schubert *et al.* (1994b) can be extended to the general case of an extended network. This idea and its implementation is elaborated in this paper.

2 METHODOLOGY

2.1 Building blocks of the hybrid network

In its simplest form a hybrid model contains two components describing two different parts of the entire process (Schubert *et al.*, 1994b). In the case of a biochemical cultivation process, for example, it is straightforward to base the model on a macroscopic mass balance formulated by means of a set of ordinary differential equations and describe the kinetics in some other way, e.g. by means of a phenomenologically obtained mathematical expression like the so-called Monod model or by means of an artificial neural network.

The alternatives of describing different parts of the model are usually not equivalent. The two possibilities of describing the kinetics in this example, are different in the sense that the Monod expression has a more global applicability, from the point of view that it can be more reliably extrapolated to substrate values not experienced during the previous experiments. On the other hand the artificial neural network is able to consider the influence of more process variables on the conversion rates, however, it provides reliable results only in areas of the state space that have been met many times during previous experiments from which the data was taken to train the network. In these areas, however, the neural network usually provides much more accurate rate values. Hence, both representations have their pros and cons. Consequently, it is of advantage to make use of them simultaneously and to combine the results they deliver. This requires a way to adequately weight both rate values.

There are different possibilities to represent a particular part of the process. In a more formal way, one can be distinguished between mechanistic descriptions, heuristic descriptions and pure process data correlations. When, as suggested, they are to be applied simultaneously, then a dynamically weighted average of their results is necessary, to obtain a single result that can be used in further calculations. Different weighting techniques have been proposed in literature:

1. Weighting using clustering techniques (e.g., Simutis *et al.* 1995, Leonard *et al.* 1992)
2. Weighting with expert systems (e.g., Schubert *et al.* 1994a)
3. Weighting based on neural networks (e.g., Haykin, 1994)

It is straightforward to combine the different representations of a particular part or aspect of the process under consideration together with the weighting module in a separate block within the entire model as schematically sketched in Figure 1. Such a block is considered the basic building block of the hybrid network model proposed in this paper.
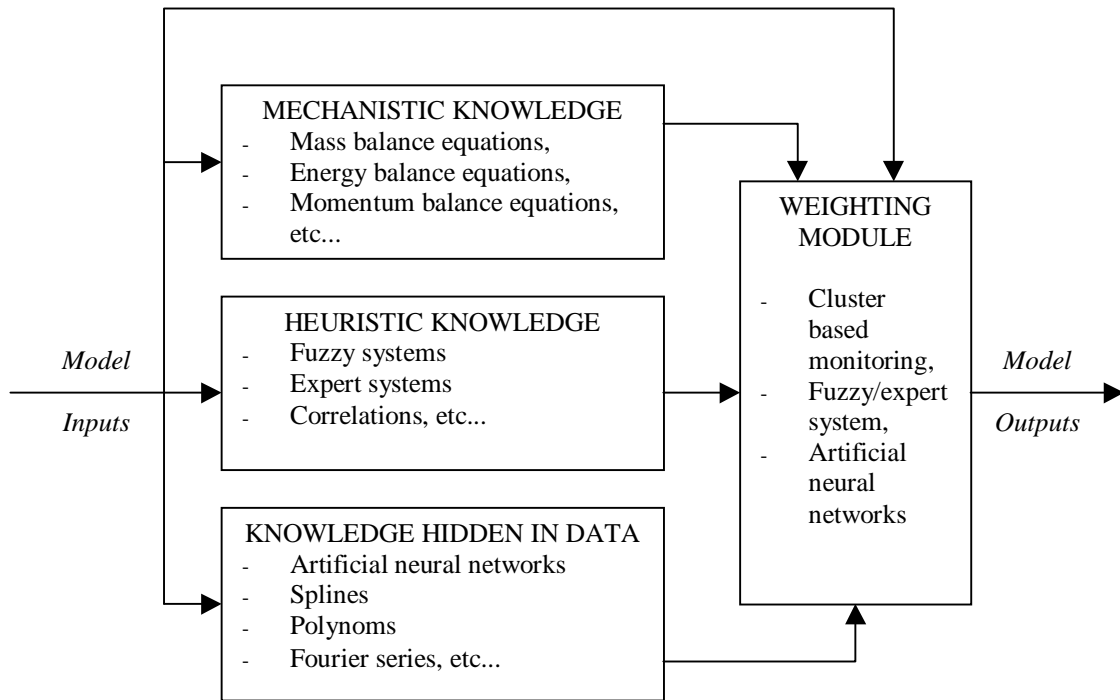
Fig. 1. Combining different ways of describing a particular part of the process. Usually, modules based on mechanistic process descriptions are combined with modules which represent heuristic knowledge and others which contain correlation-type process descriptions. Their results must be combined to form a weighted average as a unique output of this part of the entire model.

It should be mentioned that it is not necessary to incorporate all three types of knowledge representation into such a building block. For example, it obviously does not make sense to describe some part of the process, which is well investigated and for which well established mathematical models are already available, by heuristics or to learn the relevant relationships once more from process data. Hence, the extension of the blocks is different from case to case.

2.2 Composition of the hybrid network

The base of most models used in bioprocess supervision and control is a system of mass balance equations for all process components that are macroscopically changing during the process in a significant amount. The most important examples for such components are the amounts of substrates, biomass and products as well as carbon dioxide, oxygen, etc.. Particularly, one is interested in those quantities that are influencing the performance of the process under consideration, especially the amount of product developed.

Such balance equations can be formulated in a straightforward way in form of ordinary differential equations systems. There is no need to look for alternative descriptions, provided appropriate expressions can be formulated for the biochemical conversion and the relevant transport rates like the oxygen mass transfer, which are an essential part of the balance equations.

It is straightforward to regard the set of mass balance equations as the backbone of the model and represent them by a central module of the entire model. This module must be complemented by additional modules describing various different components of the mass balance equations. For example, the various transport processes, like oxygen transfer, which are of major importance in industrial production processes, since they finally limit the productivity which can be obtained with a given strain in a given bioreactor, can be described by separate modules. Of general importance are the modules for the rate expressions, which by themselves may be divided into different parts, e.g., stoichiometric relationships and kinetic relations between the various primary variables, the concentrations of the key components.

In order to work together, the modules must be interconnected. They thus can be represented by a network of modules. It is well known that such a modularization of a process model immediately enhances the transparency of the model and as such helps to avoid errors. Another essential advantage is that such a structure simplifies the practical modeling work by allowing to make use of predefined software modules that need to be adapted only slightly to fit into the model.

2.3 Parameter identification in hybrid networks

In practice, hybrid networks are used for biochemical processes of considerable complexity. The models, therefore, contain many parameters. Consequently, a considerable amount of data is required to identify the model parameters. In such cases, the computing time becomes an issue. It is a particular advantage of the hybrid networks that this parameter estimation can be performed in a very efficient way using the error-backpropagation technique. This technique applied with high advantage to train artificial neural networks can also be applied to hybrid networks, i.e. networks in which the computational nodes are of different quality and of much higher complexity than in neural networks.

Essentially, parameter estimation is an optimization problem. Usually gradient-based optimization techniques were used to tackle that problem. If the models contain strongly nonlinear features, the straightforward alternatives are the random search algorithms (e.g. Simutis and Lübbert, 1997). These will lead to correct identification results, however, they take too much computing time in extended hybrid network models. The alternative, proposed in this work, the backpropagation technique belongs to the gradient-based techniques.

As shown by Leonard and Kramer (1992), the error backpropagation technique is a very effective way to determine the Jacobian and/or Hessian matrices directly, which are determined numerically in most conventional gradient-based optimization techniques.

For a simple combination of a single neural network and a balance equation system Schubert *et al.* (1994a) already showed that this can be successfully applied to hybrid models. Here this technique is extended to arbitrarily complex hybrid models.

The mathematical details are described in the Appendix.

2.4 Practical considerations

In order to make parameter estimation of hybrid networks feasible in practice, extended software support must be provided. An example of an appropriate software package is HYBNET (Oliveira *et al.*, 1997), which contains a number of procedures which can be applied in order to design, identify and validate hybrid networks. This software already proved itself to support the employment of these model in industrial applications like process optimization, supervision, and control.

3 EXAMPLES

3.1 Biomass estimation with a simple hybrid network

The first example is a model-supported biomass estimation. The backbone of the model used is a simple mass balance equation for biomass. The second component of the model is an estimation for the specific biomass growth rate μ. As well known, the specific biomass growth rate μ can be estimated from the rates by which the gaseous reaction components O_2 and CO_2 are consumed or produced. The basic component describing the specific growth rate μ is an artificial neural network. Alternatively, an empirical relationship has been used in the model.

This component is represented by the very simple correlation proposed by Wu *et al* (1985):

$$\mu = Y_{xo} (OUR/X - m_o) \qquad (1)$$

The 2 parameters involved are the yield, Y_{xo}, of biomass produced per-oxygen consumed and, m_o, the specific amount of oxygen consumed by the yeast for maintenance purposes. A least-square minimization of the deviations between the biomass values measured off-line and the ones estimated by the correlation was used. The quasi-Newton (QN) algorithm was used with inequalty constrains defining upper and lower bounds for Y_{xo} and m_o.

Since the simple classical relationships between specific growth rate and OUR is based on assumptions about the kinetics which are not as established as the basic mass balance equation, it is straightforward to determine these relations alternatively by means of an artificial neural network, provided there is enough experimental data available to train the neural network. In the present example a simple feedforward artificial neural network was used to estimate the specific growth rate μ_{ANN} from data from carbon dioxide production rate CPR and oxygen uptake rate OUR.

As both the classical correlation and the neural network depict different globalization properties, they are used simultaneously to determine the specific growth rate μ. The question how to weight the two rates can easily be solved in this particular case: The most direct approach to weighting is the cluster technique, which attaches a weighting factor between 0 and 1 to the neural network component and the complement to one to the classical approach. The weight for the network component attached to a given point in the state space, i.e. here to a given input vector X, is determined from the number of data measured in the vicinity of X in the state space. This weight is determined by a data cluster analysis as described by Simutis *et al.* (1995).

When μ is determined, the mass balance equation for the biomass can be solved. The entire model is depicted in Figure 2.
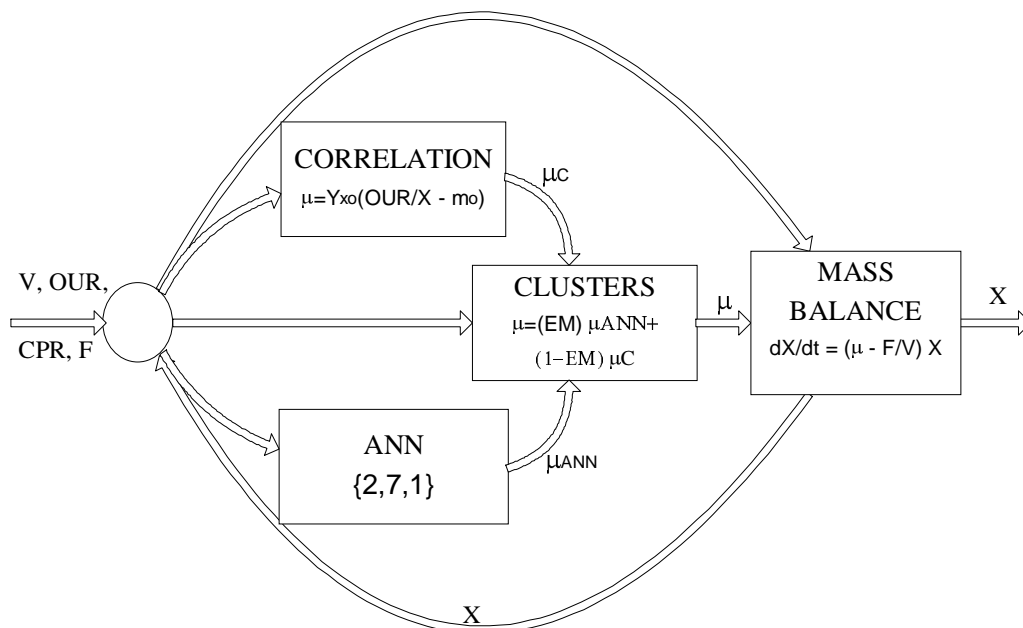
Fig. 2. Hybrid network for biomass estimation in a baker's yeast cultivation consisting of two main parts: A block containing two alternatives to determine the specific growth rate μ and a balance equation for the biomass.

In order to give an impression of the weighting procedure, typical results are shown in Figures 3 through 4. Figure 3 shows the output μ_{ANN} of the neural network for the specific growth rate as a function of the corresponding input variables OUR and CPR. As the experiments deliver measurement data pairs for these variables in a narrow area of the (OUR, CPR)-space only, we can consider μ_{ANN} reliable only in a small region as shown in Figure 4 by means of the evidence measure EM, which is a normalized indicator of the measurement information available for the different (OUR, CPR)-pairs.
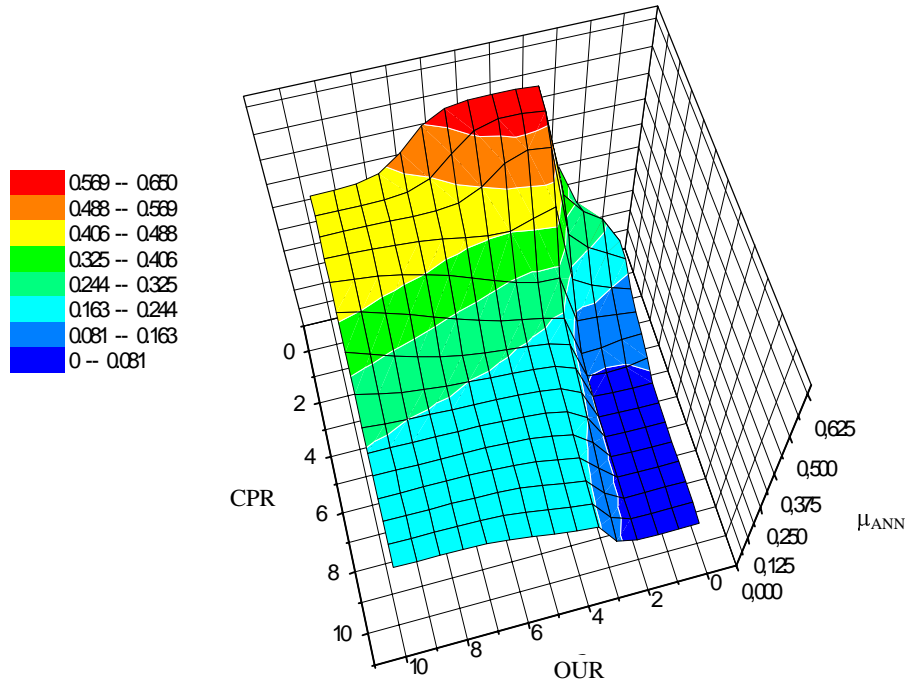
Fig. 3. Pattern of the specific growth rate µANN as determined from the neural network component of the hybrid network for a baker's yeast cultivation process from the input variables OUR and CPR.
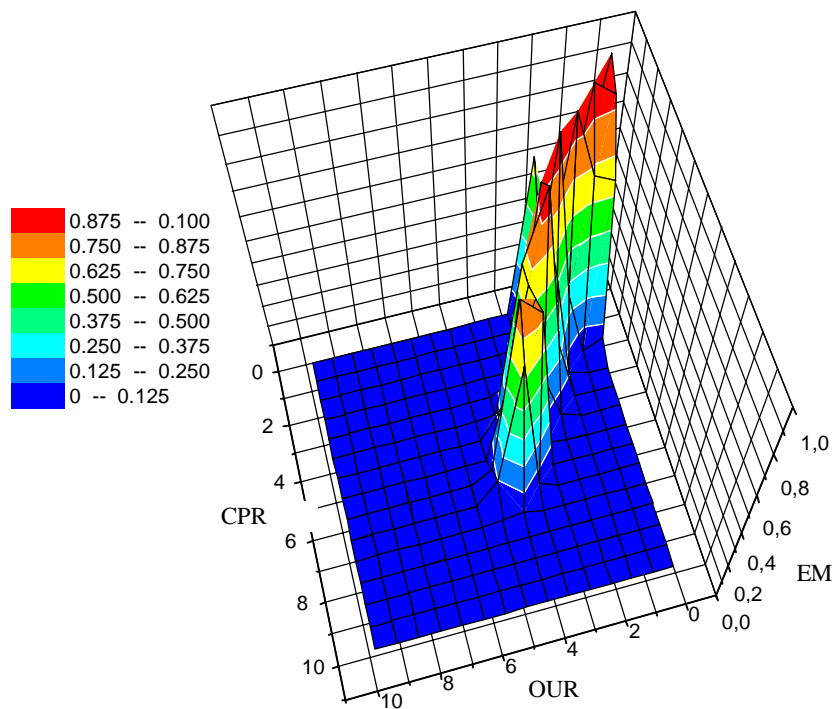

Fig. 4. Extrapolation measure EM characterizing the evidence of the neural network component of the hybrid network from OUR and CPR data measured during a set of baker's yeast cultivation processes

EM is used to determine the weight of the neural network component in the block used to determine μ. The weight of μC, the result of the correlation, is then 1-EM. The resulting μ as a function of time is depicted together with the estimation X(t) in Fig. 5.
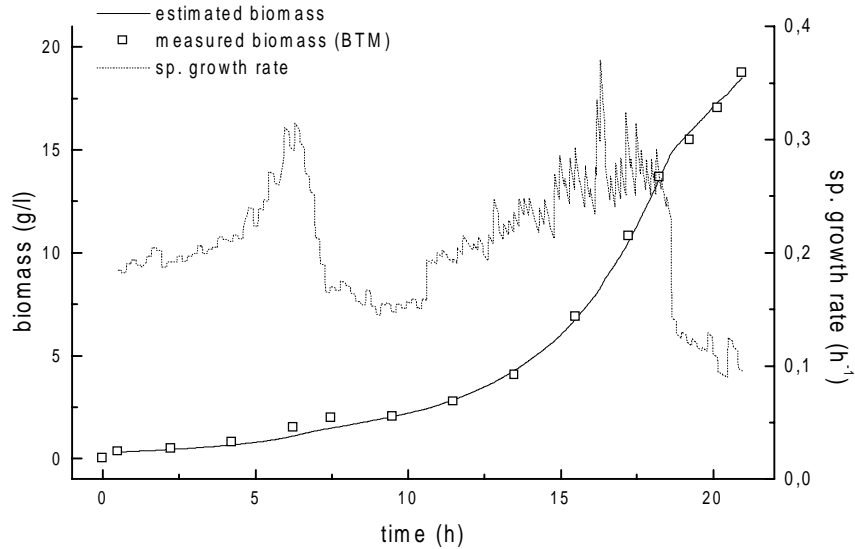
Fig. 5. Typical results of biomass estimates X and estimates of the specific growth rate μ. The latter is the result of the weighted average between the two estimates μ_{ANN} and μ_C. The symbols are measurement data from a representative experiment at which the estimator was used.

The estimation results show a fairly high fluctuation in the estimation of the specific growth rate but a rather good estimation quality for the biomass X.

The biomass estimation procedure was validated with the cross validation procedure using data records from 3 cultivations not used during the parameter identification procedure. The identification itself was performed with data records from 7 cultivations.

In order to demonstrate the practical advantage of the error backpropagation technique for the parameter estimation procedure, a comparison was performed between the conventional approach of parameter estimation using gradient techniques (second order gradient methods with numerical evaluation of the Jacobian and Hessian matrices) and the error backpropagation technique described previously. The mean square estimation error MSE as a function of the computing time CPU is depicted in Figure 6. This figure shows the evolution of the MSE until a pre-established error value is achieved.
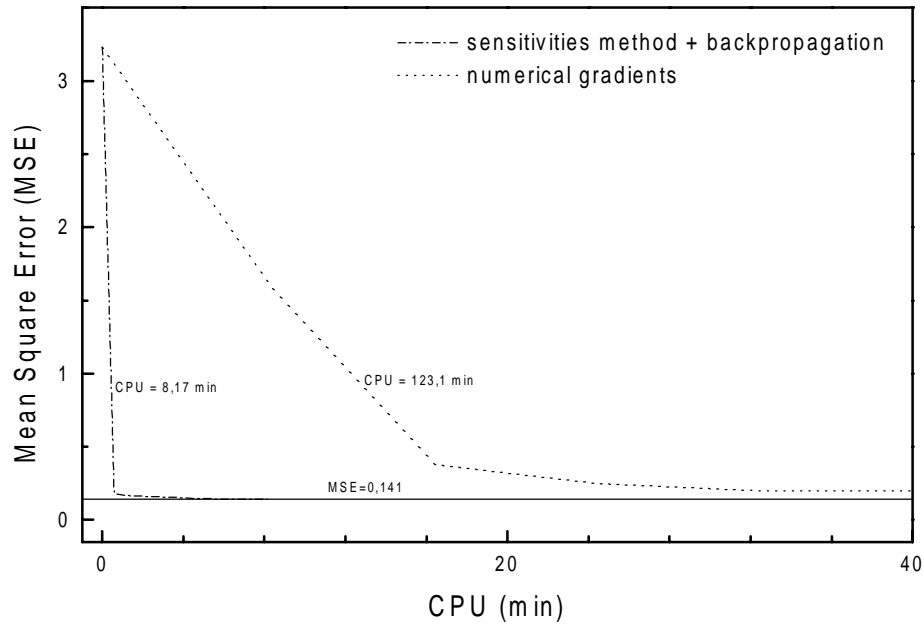
Fig. 6. Mean square estimation error MSE as a function of the computing time CPU required to reach a pre-established error value. These results were obtained with a 233 MHz Alpha Station (Digital Equipment Corp.) using data from 7 cultivations.

3.2 Feed rate profile optimization for baker´s yeast production

In baker's yeast production, the aim is to produce within a given production time t_f as much yeast biomass [kg] as possible. This process is operated as a fed-batch process in practice. Hence, the objective function J, which has to be optimized by means of an appropriate feeding rate profile F(t) and related start values of the state variables is:

$$J = \frac{x(t_f)V(t_f)}{t_f + t_p} = \frac{\text{Biomass [kg]}}{\text{Total Batch Time [h]}} \tag{2}$$

where t_p is the time required between two successive production runs to prepare the reactor for the next batch.

The first step of such a feed rate optimization is the identification of the relevant process model, which was formulated as a hybrid network sketched in Figure 7.
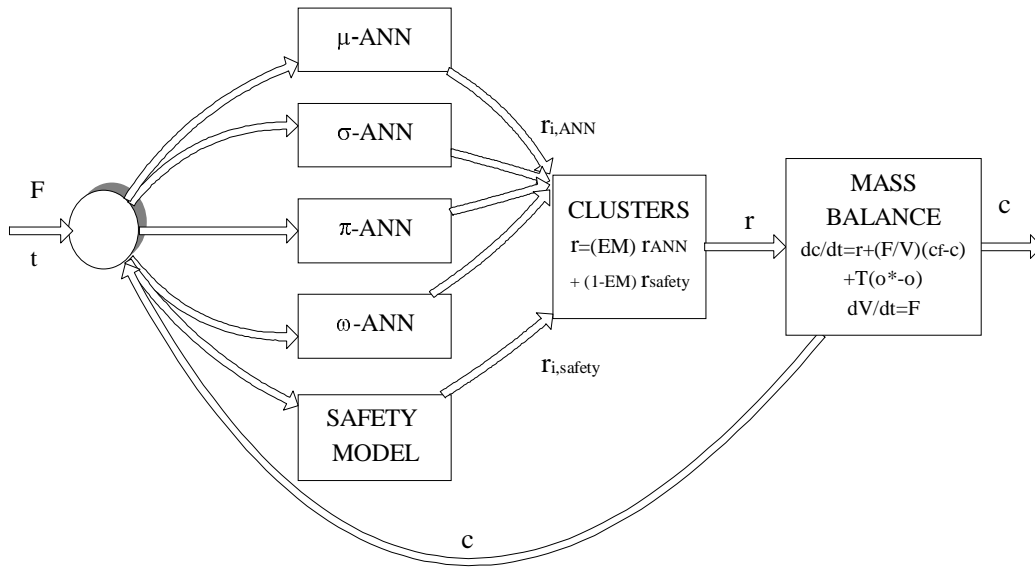
Fig. 7. Hybrid network used during a feed-rate optimization for a baker's yeast production process.

As in the foregoing example, the basis of the model is a mass balance. For the four concentrations that significantly change during the cultivation and for the volume, we obtain the following ordinary differential equation system

$$\frac{dc}{dt} = r\, x + \frac{F}{V}(c_f\text{-}c) + T(o^*\text{-}o) \tag{3a}$$

$$\frac{dV}{dt} = F \tag{3b}$$

with the concentration vector $c = [x, s, e, o]^T$ for biomass, substrate, ethanol and oxygen and the absolute rate vector $r = [\mu, \sigma, \pi, \omega]^T$, μ, σ, π, ω being the corresponding specific rates. $T = [0, 0, 0, k_L a]$, where $k_L a$ is the oxygen mass transfer coefficient.

The specific rate expressions for μ, σ, π, ω representing the biochemical conversion process are described by different artificial neural networks. From experience with such systems, this modularization is most often of advantage in modeling such systems (e.g. Haykin, 1994). When there is enough training data, then artificial neural networks proved to be very good models for the process kinetics. At least they can be built at the best benefit/cost ration.

In the example reported here, data sets from 10 fermentations performed in the same region of the state space were available. In this case, this proved to be enough to train a reliable neural network for the three specific rate expressions μ, σ, π, ω. It should be stressed, that the quality of the representation of the specific rate is much dependent on

the quality of data and the complexity of the kinetics. There is no general rule about the amount of data required for the training of the networks.

Nevertheless a classical model must be employed to make sure that we can also provide a sufficiently reliable rate when the state vector runs out of the region in the state space in which we have enough measurement evidence. The corresponding module can be considered a safety module. The safety model used in this example is a classical model based on simple Monod expressions. Equations similar to those developed by Sonnleitner and Käppeli (1986) were used. The parameters were fitted to the same data as were used to train the neural network.

The weighting of the two specific rate vectors was performed in the same way as in example 1. For all the specific rates produced by the four neural network components the same weights were used. The complement to one was taken as the weight for the conventionally obtained specific rate vector.

Once the model is identified it can be used to perform the very task it had been designed for, the feed rate optimization. In order to perform this optimization task, a convenient form of representation of the feeding profile F(t) must be chosen. While most often a polynomial approach was favored (e.g. Montague and Ward, 1994), we took the more flexible choice of an artificial neural network to represent this nonlinear profile. As such, a simple feedforward network with time as input, 5 hidden nodes and with F as single output was chosen.

Now the task is to optimize the parameters of the feeding profile, i.e. the weights in neural network used to describe F(t) as well as the final fermentation time t_f, such that the performance criterion J is maximized. For the initial volume v_o and the initial substrate concentration s_o typical fixed values for the cultivation equipment were used. The constraints under which the optimum must be found are:

$v_{max} = 13$ L
$F_{max} = 0.35$ L/h

The result obtained under these conditions are depicted in Figure 8.

A characteristic feature of this result is that it allows the formation of a considerable amount of EtOH during the fermentation in order to keep the biomass production rate at a high value. In the second half of the production this EtOH is then consumed in order to keep the overall substrate conversion into biomass close to 100%.
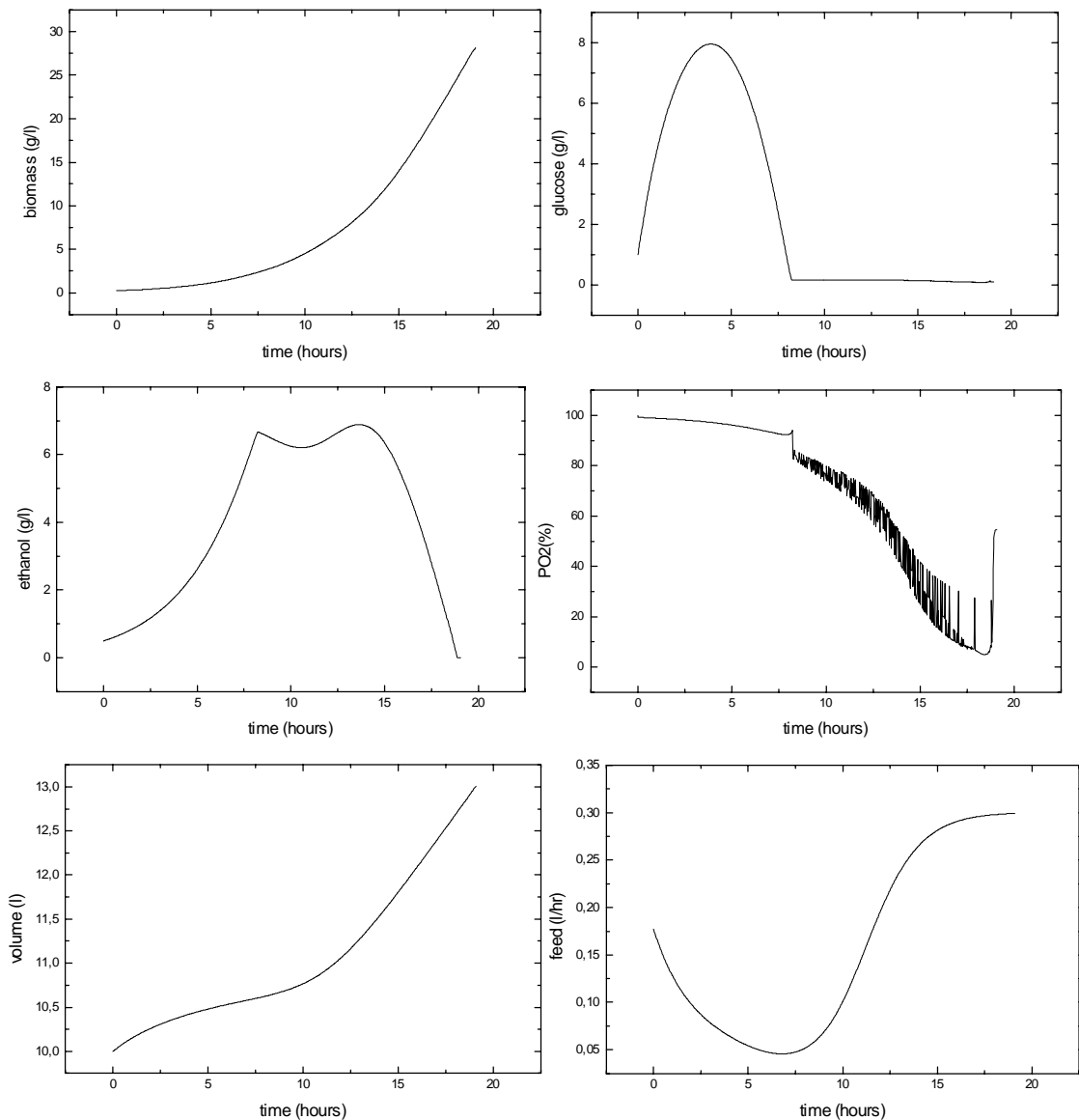
Fig. 8. Typical results of the described feed rate optimization procedure. The profiles of the feeding rate F is shown together with the corresponding profiles of the state variables biomass, substrate, ethanol, and oxygen (pO2) concentrations as well as the volume development.

4 CONCLUSIONS

Hybrid networks can be composed of modules that represent parts or aspects of a complex biochemical production process on different levels of sophistication. The key advantage of hybrid networks is that they allow to make use of a much larger knowledge base to construct process models. Hence, it is to be expected that the models more accurately describe those features of the process that are relevant to the task to be solved. The resources that can be exploited are not only the well understood mechanisms but also the wide area of the heuristics accumulated in the production plants as well as the data records from many production runs.

As a modular representation of the process hybrid networks generally have the further advantage to keep the process model highly transparent. With modular models it is easier to detect and thus avoid modeling errors. Also, with modular models it is easier to maintain the models and to adapt them to the ever changing conditions in production plants.

A decisive advantage of hybrid models is that, when their outputs can be represented as continuous and differentiable representations of their inputs, the backpropagation method well known from the training of simple neural networks can be used to significantly reduce the computational requirements for model identification. This is of high importance to models that contain several artificial neural network components, as such components usually have many parameters and the corresponding identification takes much time.

The hybrid modeling technique already proved to be of high value in industrial applications.

APPENDIX - SENSITIVITY ANALYSIS OF HYBRID NETWORK MODELS

Here we discuss a general sensitivity analysis with respect to hybrid network models of biochemical production systems. Let us assume that the system can formally be described by the ordinary differential equation system

$$\frac{dX}{dt} = f(P, X, U, t) \qquad \text{(A-1a)}$$

$$Y = g(P, X, U, t) \qquad \text{(A-1b)}$$

with initial conditions,

$$X(t_0) = X_0 \qquad \text{(A-1c)}$$

where X is the vector of state variables, Y a vector of measurement variables, U a vector of external influence variables, P a vector of model parameters and t the independent variable time. This assumption means that the network represents a continuous relationship between the state variables X as well as between the state variables and the measurement variables Y.

Since a hybrid network is a network of computational nodes we additionally need a general model description of the nodes. We assume for an arbitrary node k that its vector Y_k of output variables is dependent on a vector of inputs Z_k. This dependency is defined by a set of continuous and differentiable equations H_k containing a set of parameters that can be represented by the vector P_k

$$Y_k = H_k(Z_k, P_k) \qquad \text{(A-2)}$$

By sensitivities we mean the output/input and output/parameter sensitivity matrices:

$$\frac{\partial Y_k}{\partial Z_k} = G_k(Z_k, P_k) \qquad \text{(A-3a)}$$

$$\frac{\partial Y_k}{\partial P_k} = E_k(Z_k, P_k) \qquad \text{(A-3b)}$$

These sensitivity expressions are not only evaluable for conventional equation systems but also for the input-output relationships on which the other main module types of hybrid networks are founded, in particular fuzzy rule systems and artificial neural networks:

Fuzzy systems. If all the membership functions of all the fuzzy sets in the fuzzy rule system are continuous and differentiable, the rule-based system can be mapped into a set of continuous and differentiable relationships H_k (Wang 1994). Thus, the

relationships G_k and E_k can be derived from H_k. An example of such fuzzy system is discussed in detail by Simutis *et al.* (1995). The parameters P_k involved define the form and position of the membership functions. The requirements can easily be fulfilled when the membership functions of the fuzzy variables are chosen to be Gaussian bell-type curves. Then the parameters are the means and standard deviations of the Gaussian curves.

Artificial neural networks. All the types of ANNs which can be trained with the backpropagation technique meet the requirements for the nodes stated before. The sensitivities defined by eqns. (A-3a) and (A-3b) can be computed with the error backpropagation algorithm. The parameters P_k are the weights in the network connections.

Obviously, any linear combination of modules of type continuous and differentiable equations, fuzzy system and artificial neural network which meet these requirements also can be taken as a node. Thus, a more complex nested networks can be constructed with these basic types of nodes.

When we deal with a network consisting of a number of interconnected nodes then we first need a description of the connections. These can be defined by four-dimensional matrix $W=[w_{i,j,k,l}]$ in the following way:

$$w_{i,j,k,l}= \begin{cases} 1 & \text{if the connection exists} \\ 0 & \text{if the connection doesn't exist} \end{cases} \qquad \text{(A-4)}$$

where the element $w_{i,j,k,l}$ defines the connection between output j of block i with input l of block k>i.

Then the inputs to block k can be computed in the following way

$$z_{k,l} = \sum_{i=1}^{k-1} \sum_{j=1}^{\dim(Y_i)} w_{i,j,k,l} Y_{i,j} \qquad l=1,\ldots,\dim(Z_k) \qquad \text{(A-5)}$$

The output of the same node can then be determined by evaluating using eqn (A-2).

Now, we come back to the initial representation (A-1) of the entire system. A partial differentiation of these equation after the parameter vector P and the vector U leads to the following equations

$$\frac{d}{dt}\left(\frac{\partial X}{\partial P}\right) = \frac{\partial f}{\partial X}\frac{\partial X}{\partial P} + \frac{\partial f}{\partial P} \qquad \text{(A-6a)}$$

$$\frac{\partial Y}{\partial P} = \frac{\partial g}{\partial X}\frac{\partial X}{\partial P} + \frac{\partial g}{\partial P} \qquad \text{(A-6b)}$$

$$\frac{d}{dt}\left(\frac{\partial X}{\partial U}\right) = \frac{\partial f}{\partial X}\frac{\partial X}{\partial U} + \frac{\partial f}{\partial U} \qquad \text{(A-6c)}$$

$$\frac{\partial Y}{\partial U} = \frac{\partial g}{\partial X}\frac{\partial X}{\partial U} + \frac{\partial g}{\partial U} \qquad \text{(A-6d)}$$

They can be identified as differential equations for $\partial X/\partial P$ and $\partial X/\partial U$ and form the base of the *sensivity method* (e.g., Frank, 1978). To solve these equations with the initial conditions

$$\left(\frac{\partial X}{\partial P}\right)_{t=t_0} = 0 \qquad\qquad\qquad (A\text{-}6e)$$

$$\left(\frac{\partial X}{\partial U}\right)_{t=t_0} = 0 \qquad\qquad\qquad (A\text{-}6f)$$

one needs the matrices $\partial f/\partial X$, $\partial f/\partial U$, $\partial f/\partial P$, $\partial g/\partial X$, $\partial g/\partial U$, and $\partial g/\partial P$. They can simply be determined with the well known backpropagation procedure (Rumelhart et al 1986) applied to the overall hybrid network structure. Notice that the principles followed are similar to error backpropagation in neural networks. The main difference is that the classical sigmoid functions, usually employed in the single nodes in neural networks, are replaced by the function $Y_k=H_k(Z_k, P_k)$ (eqn. A-2) and the respective gradients defined by eqns. (A-3).

REFERENCES

Feyo de Azevedo, S., B. Dahm, F.R. Oliveira (1997) "Hybrid Modelling of Biochemical Processes: A comparison with the conventional approach," *Computers chem. Engn*, **21**, Suppl., pp. 751-756

Frank, P.M. (1978). Introduction to System Sensivity Theory. Academic Press, New York

Gupta, M.M. and D.H. Rao (1994). On the principles of fuzzy neural networks. *Fuzzy Sets and Systems*, **61**, pp. 1-18

Haykin, S. (1994). Neural Networks: a Comprehensive Foundation. Macmillan. New York

Leonard, J.A., M.A. Kramer, L.H. Ungar (1992). A neural network that computes its own reliability. *Comp. Chem. Eng.*, **16**, pp. 819-835

Lin, Chin-Teng and C.S. George Lee (1991).Neural-Network-Based Fuzzy Logic Control and Decision System. *IEEE Transactions on computers*, **40(12)**, pp. 1320-1336

Montague, G.A., A.C. Ward (1994). A Sub-optimal Solution to the Optimisation of Bioreactors Using the Chemotaxis Algorithm. *Process Biochemistry*, **29**, pp. 489-496

Oliveira, R., R. Simutis, S. Feyo de Azevedo, A. Lübbert (1997). HYBNET, an Advanced tool for Process Optimization and Control. (in press)

Psichogios, D.C. and L.H. Ungar (1992). A hybrid neural network - First principles approach to process modelling. *AIChE J.*, **38**, pp. 1499-1511

Rumelhart, D.E., G.E. Hinton, R.J. Williams (1986). Learning internal representations by error backpropagation. In *Parallel Distributed Processing*, **1** (D.E. Rumelhart, and J.L. McClelland, Eds). MIT, Cambridge, MA

Schubert, J., R. Simutis, M. Dors, I. Havlik, A. Lübbert (1994a). Hybrid Modelling of Yeast Production Processes – Combination of a priori knowledge on Different levels of Sophistication. *Chem Eng. Technol.*, **17**, pp. 10-20

Schubert, J., R. Simutis, M. Dors, I. Havlik, A. Lübbert (1994b). Bioprocess optimization and control: Application of hybrid modelling. *Journal of Biotechnology*, **35**, pp. 51-68

Shi, Z. and K. Shimizu (1992). Neuro-fuzzy control of bioreactor systems with pattern recognition. *J. Ferment. Bioeng.*, 74, pp. 39-45

Simutis, R., I. Havlik, F. Schneider, M. Dors, A. Lübbert (1995). Artificial Neural Networks of Improved Reliability for Industrial Process Supervision. Preprints of the 6th Int. Conference on Computer Applications in Biotechnology, Garmisch-Partenkirchen, Germany, pp. 59-65, May 14-17, 1995

Simutis, R., Lübbert, A. (1997) „A comparative study on random search algorithms for biotechnical process optimization," *J. Biotechnol,* **52**, pp. 245-256

Sonnleitner, B., O. Käppeli (1986) "Growth of Saccharomyces cerevisiae is controlled by its limited respiratory capacity: Formulation and verification of a hypothesis," *Biotechnol. Bioeng.*, **28**, pp. 927-937

Thomson, M.L., M.A. Kramer (1994) "Modeling chemical processes using prior knowledge and neural networks," *AIChE J.*, **40**, pp. 1328-1340.

Wang, L.-X. (1994). Adaptive Fuzzy Systems and Control: Design and Stability Analysis. Prentice Hall, Englewood Cliffs, N.J. USA

Werbos, P.J. (1992). Neurocontrol and Fuzzy Logic: Connections and Designs. *International Journal of Approximate Reasoning*, **6(185)**, pp. 185-219

Chapter 3

HYBNET, an Advanced Tool for Process Optimization and Control

Abstract. HYBNET is a software package that supports advanced model supported process design and operation. HYBNET stands for HYBrid NETwork, since it is able to compose the process model in a modular way and allows to connect these nodes in form of a network. It is hybrid in different respects: First, the models representing the different parts of the process or its behavior in different situations can be formulated at different levels of sophistication, corresponding to the knowledge available about that particular aspect of the process. In most applications the modules are formulated in different representations like neural networks, fuzzy rule-based models and classical differential equations. More than a hundred of such different nodes can be interconnected in a most flexible way in order to form a well performing process model as the base for systematic process optimization, process supervision and closed-loop control. The nearly arbitrarily structured models can be identified in roughly the same way as the training of artificial neural networks, namely by means of error backpropagation schemes. One of the most essential advantages from the practical point of view is that the software package is nearly platform independent. It only requires a link, e.g. with a TCP/IP protocol, to the process control computer used. The concept of the software is illustrated at the example of a baker's yeast production process.

1 INTRODUCTION

There are numerous proposals recorded in literature to improve the performance of biochemical production processes by modern model supported techniques like feeding profile optimization or closed loop control. However, the industrial acceptance was rather poor. The computer hardware available at production processes is most often used for data acquisition and low level control only. One of the main reason is that the benefit/cost ratio for advanced techniques, which is dominated by the development cost and very often additionally by the cost to adapt those systems to the ever changing conditions in a real production plant, was insufficient. Here, we propose a software package, in which many of the obstacles were removed.

Process design, supervision, and closed loop control is and will be the central activity of bioprocess engineers. In industrial practice, there is no reason to proceed differently to the way in which good scientific work is being performed. Usually, the first approach is to collect and structure the knowledge about the process under consideration. This results in a system of interacting partial processes. The straightforward way to proceed, is to characterize the behavior of all that elements with respect to their importance in terms of the objectives of the particular task to be solved. Finally, the entire process, which can be viewed at as a network of all its interacting components can be optimized utilizing this detailed knowledge. Any practical optimization of a real production process must be based on predictions of the process behavior. This requires a model that can be exploited numerically. Thus, models for the different process components and dynamical descriptions of their mutual interactions become necessary, and the entire model can be considered a network of interacting software modules.

A major obstacle to putting this general and well established idea into industrial practice is missing software support for processes, which are as complex as most industrial biochemical production processes. Interactions of many different subsystems must be considered and often, the models for the different sub-processes cannot be formulated on the same high level of understanding. Thus, we propose a hybrid approach, which means an application of a mixture of models on different levels of sophistication and scrutiny. For instance, the mass balance may be formulated with crisp mathematical equations, the kinetics in a data driven way with neural networks and, at the same time it becomes possible to make use of rules-of-thumb where no data or models are available by using fuzzy rule systems. In such a way it is not only possible to formulate the models on the level of corresponding knowledge available (thus avoiding transfer losses) but also to adapt these representations to the needs with respect to the task to be solved (to increase the benefit/cost-ratio).

2 CONCEPTUAL FRAMEWORK

2.1 Guidelines for the development

The central aim is to provide a tool which helps to solve typical tasks in bioprocess engineering, e.g., in designing new processes or in optimizing existent ones. The

general approach is a model supported approach assuming that the particular tasks can be solved the better and faster the more relevant knowledge can be activated.

HYBNET provides all tools required to formulate, identify and process hybrid process models (cf. Fig. 1) as described by Schubert et al. (1994) and Simutis et al. (1996). However, it is worth to note, that instead of modeling, the focus is on solving particular tasks of process engineers like optimizing the process performance. The process model is not considered to possess a value by itself. It is thus rated by the advantage it provides for solving the task.
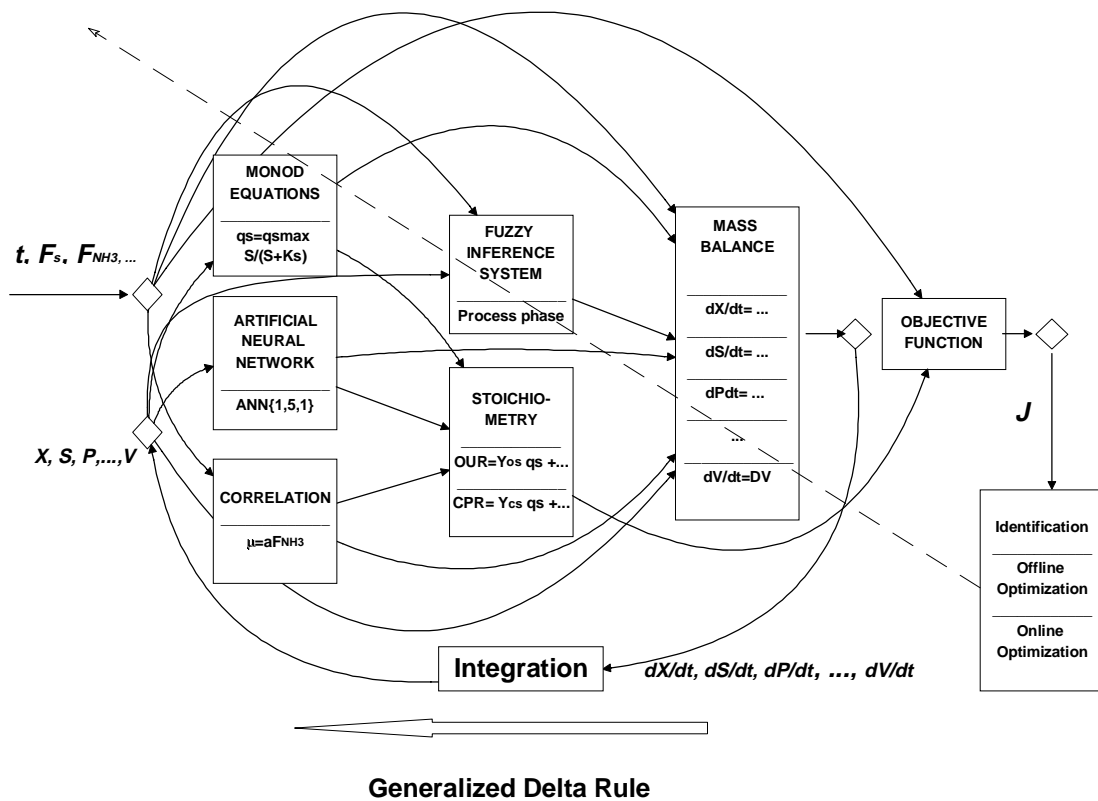
Fig. 1. HYBrid NETwork general structure

The essential objective of HYBNET is to provide a general software package, which contains all the tools necessary to solve typical tasks of process optimization and control. HYBNET is designed to serve not only for the software development, but also for its maintenance and extension in the course of a process. For instance, whenever new data become available in a process running under its control, HYBNET allows to semi-automatically make use of the data for improving the knowledge base it uses to improve the process performance. From these general aims it becomes clear, that the application of HYBNET first requires a quantitative definition of what is meant by process performance. A typical objective might be to improve the process performance by reducing the variance in some quality characteristics of the product by means of a better control.

HYBNET also provides a variety of tools which are necessary to make its application more convenient. E.g. process data acquired by some front end processor can be taken over, preprocessed and visualized under different aspects. This allows a higher level process supervision. Off-line measured data can be made available etc.

2.2 Main components required

The following toolboxes are required within the HYBNET software package

1. One for formulating hybrid process models
2. Another for data preprocessing, conditioning and storage
3. A third for optimization of complex process models
4. A further one for links to the process and process control

Additionally, much effort must be placed into a friendly user interface, and, last but not least, a managing system is required that synchronizes the different activities required to perform the particular task

3 MAIN COMPONENTS OF HYBNET

3.1 Formulation of hybrid models

A wide variety of tools are made available by HYBNET in order to construct, parameterizes and test hybrid process models. A general scheme is given in Fig. 2.
Module *xhybrid* support users activities in hybrid modeling by supporting him during the phase of model structure definition and in parameter identification procedures. It does not only manage the development of the basic process model but also the applications of the model for the particular task.

Data management

Data build the backbone of a process control system, hence they must be carefully taken up, saved and processed. There are many facets that must be considered:

1. Direct access to all data relevant to the task to be performed. This is guaranteed by means of a real time data base, which holds all data relevant to the task under consideration and which might be of importance to solve it properly. This data base is directly placed within the working memory of the computer, so that a quick random access is possible.
2. Data transfer between the front end processor that performs the data acquisition and low level control directly at the plant equipment. Most often this transfer is being performed via an network (local or remote) and processed via an TCP/IP-protocol. This data transfer is used to take over the measurement data, and to transmit the setups for the local controller back to the controllers running in the front end processor. The transfer must be sufficiently fast in order to allow model supported

measurements of key process variables that cannot be measured directly or which must be supervised by fault detection algorithms running under HYBNET.
3. Communication with the user via an appropriate user friendly interface, which guides the user in all situations where he does not take the initiative. Data which characterize the current state of the process or its simulation are displayed in a form which allow a quick overview over the current situation.
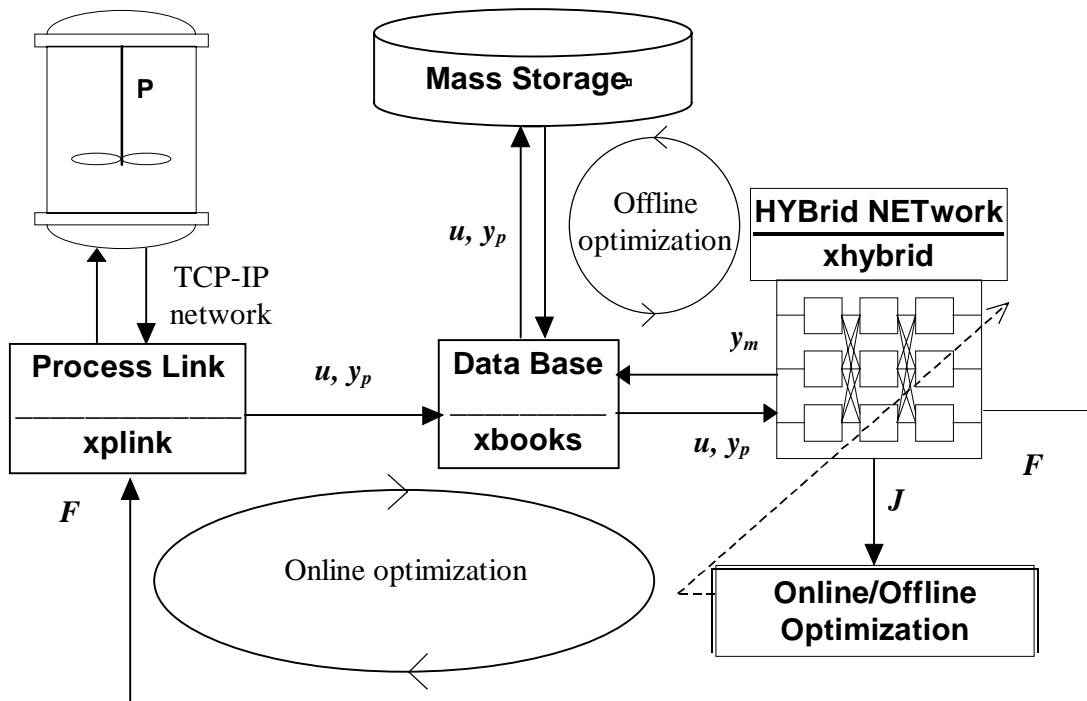

Fig. 2. Interactions between HYBNET main software components and the process.

An issue which is of major importance to applications of data management software in biochemical production processes is that in these processes a lot of measurement are made off-line in the analytics laboratory. In on-line applications it is essential to incorporate these measurement at the earliest time. Once the data are known to the system, it must be used to improve the knowledge about the state of the process and possible consequences must be considered and drawn when necessary.

Although data management is widely considered a non-attractive task for scientists and thus under-emphasized in literature, it is becoming well recognized by specialists to be one of the most important aspects to be considered when it comes to build an effective process model and to a reduction of the number of experiments during process design and optimization.

In HYBNET, the module *xplink* is responsible for the on-line and off-line communication with the process front end controller systems. The standard protocol provided is TCP/IP, however, different techniques are possible.

The real time data base is controlled by the module *xbooks*. It also manages data pre-processing and the visualization.

3.3 Optimization

Process optimization is one most prominent task to be performed by a supervisory system like HYBNET. There are several tasks where optimization is the critical issue:

1. Model parameter identification from process measurement data is one example, where the optimization is aiming at a minimization of the root mean square error between the process measurement data and the model predictions of the measurement signals.
2. The second aspect which is of primary interest is optimization of the control variable profiles and start parameters like volume and concentrations of various components in cultivations in a open loop fashion.
3. The third important aspect considered in HYBNET is the on-line optimization of the manipulated variables over short time horizons.

There are several routines available in HYBNET for the different aspects. They range from classical backpropagation (Rumelhart et al. 1986, Werbos 1990) routines used to train single artificial neural networks to several different random search techniques which are of general use (Simutis and Lübbert 1997).

3.4 Closed-loop control

xhybrid was said to be the module that supports hybrid model development, but it can do more. It can also be used to define and parameterize controllers in roughly the same way as model development: by definition of the control system structure and by the corresponding parameter identification. It considers the controller to be one additional process component.

3.5 Cooperation with commercial software tools

It does not make sense to perform tasks with a new software, where well established software tools are already available. Hence HYBNET was designed as an open software which allows to make use of software packages, which are widely distributed in industry, like Matlab, Scilab, Excel, Origin, etc. For instance, tasks that can conveniently performed by Excel should be performed with this tool.

4 EXAMPLES OF TASKS THAT CAN BE SOLVED WITH HYBNET

To give some examples of the quality of the tasks that can be solved with HYBNET, we will discuss some questions appearing during the optimization of a yeast production

process. The process performance *J* is assumed to be mainly influenced by the mean specific growth rate, which is tried to keep on the desired level μ_d and the biomass/substrate-yield $Y_{x/s}$:

$$J = Y_{x/s} - \lambda\,(\mu_d - \mu) \tag{1}$$

where λ is a weighting factor determining the strength of the penalty considered when μ is deviating from μ_d. One main constraint is a maximum fermentation time t_f. The objective is to maximize the performance *J*.

The straightforward way to develop an improved process optimization and control strategy is to first find the most important characteristics of the process with respect to the objective function *J*. Then, profiles of the manipulatable quantities most significantly influencing the objective function are determined. This is the off-line optimization step (also called open loop control). Finally, a closed loop controller is developed in order to keep the process on its predefined optimal path. When this is not accurately enough possible, corrections determined from the measured and the estimated state of the process, can be proposed by HYBNET

4.1 Feature identification

According to Sonnleitner and Käpelli (1986) the primary biological feature ruling the biomass growth in a yeast production is the maximal respiratory capacity $q_{c\,max}$ of the strain used. While in continuous cultures, this feature is roughly stationary, it must be assumed to be time dependent in real fed-batch production processes. Hence, the first task is to identify the time variance of this key quantity making use of the available data from the process under consideration.
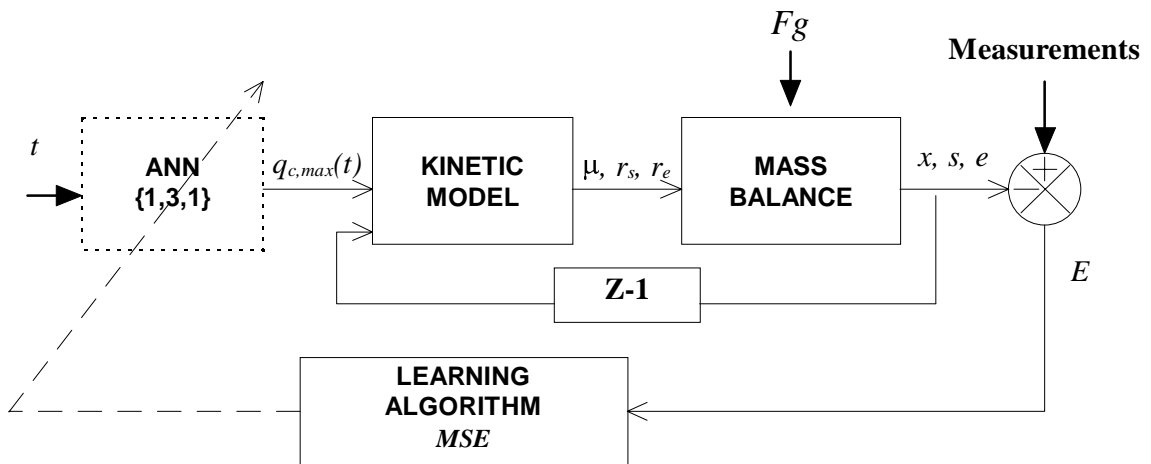

Fig. 3. Hybrid network for feature identification. An artificial neural network ANN{1,3,1} is used to identify the function $q_{c,max}=f(t)$. Learning algorithm: Backpropagation+Cross-validation+Conjugate gradients with line search+Bacth MSE objective function.
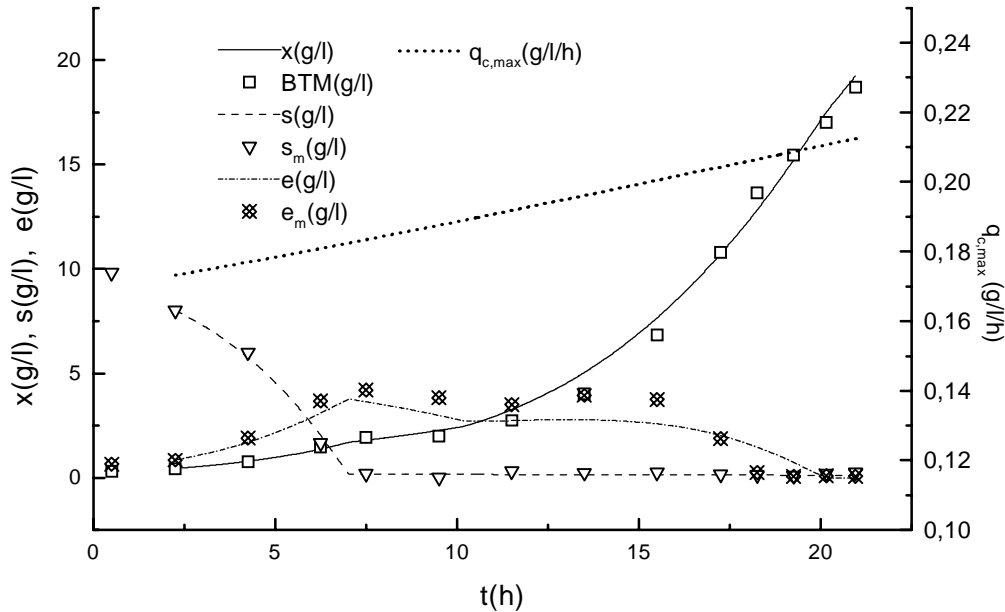
Fig. 4. Feature identification: profiles of biomass (x-estimated, BTM - measured), glucose (s-estimated, s_m measured), ethanol (e-estimated, e_m-measured) and feature ($q_{c,max}$).for a typical fermentation.

The hybrid approach is: The back bone of the model used is a set of mass balance equations for biomass x, substrate s and metabolic product ethanol e. In this simple example, the relevant yields coupling the mass balance equations are based on a simple stoichiometry. The kinetics containing the feature 'maximum respiration capacity' is based on the Monod expression. Since there is no a priori knowledge about $q_{c\ max}$, it is straightforward to use a neural network as a general form to describe this time dependence. As shown in Fig. 3, the problem can be understood as finding $q_{c\ max}$ in an optimization procedure minimizing the residuals between experimental data and the prediction by the model. HYBNET allows to use several optimization algorithms to solve that problem. A typical result is shown in Fig. 4.

4.2 Open-loop control

When the time behavior of $q_{c\ max}$ is known, then it is an easy task to determine the optimal feeding strategy for the glucose with respect to the predefined objective function. In the example, we do not proceed the conventional way of open loop control of determining the feedrate profile $F(t)$. Instead, we concentrate on the quantity more directly describing the substrate uptake of the organisms. This is the specific substrate consumption rate q_s and we are thus looking for an optimal profile $q_{s\ opt}(t)$ of this key quantity.

Since the objective function is usually not simple and the model rather complicated due to its non-linearity, classical gradient procedures do not always work properly.

For such situations, HYBNET provides several random search methods to find the optimum profile (Simutis and Lübbert 1997). The scheme of the optimization procedure is shown in Fig. 5. The resulting $q_{s\ opt}(t)$, shown in Fig. 6, was obtained with an algorithm based on an evolutionary programming technique (Simutis and Lübbert 1997).
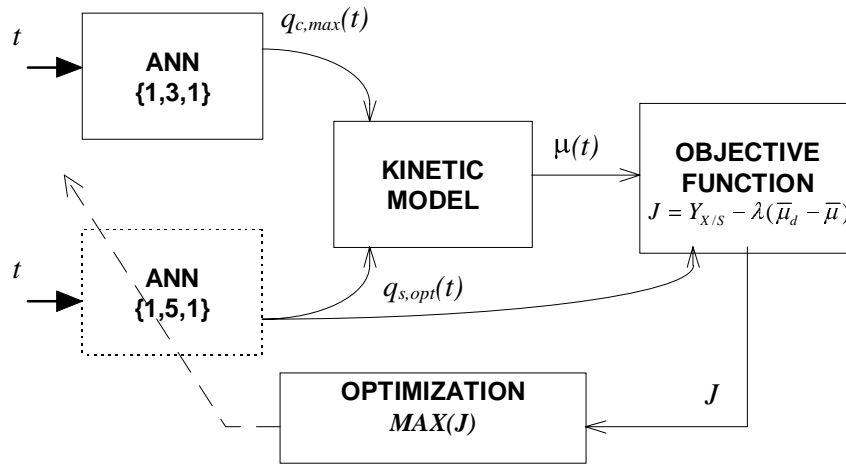
Fig. 5. Hybrid network for offline optimization (ANN trained with an evolutionary programming algorithm)
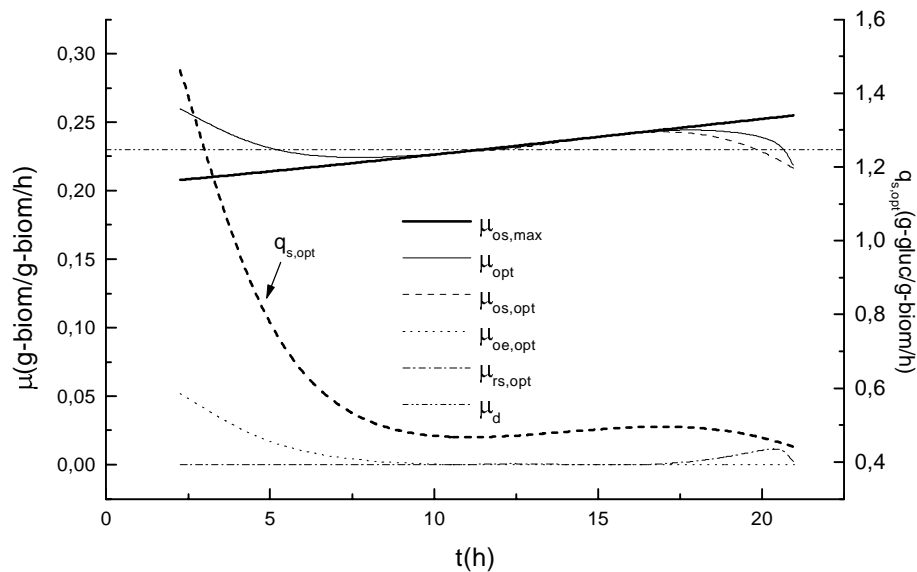
Fig. 6. Offline optimization results: the desired value for the growth rate is 0,23 g-biom/g-biom//h. The obtained yield was Yx/s=0,489. $\mu_{os,max}$ is the maximum growth rate for the glucose oxidation pathway, μ_d is the desired value, μ_{opt} is the estimated optimal profile, $\mu_{os,opt}$, $\mu_{oe,opt}$ and $\mu_{rs,opt}$ are estimated profiles for glucose oxidation, ethanol oxidation and glucose reduction respectively.

4.3 On-line control

Once a profile for the key manipulatable quantity, in our example the optimal specific substrate consumption rate $q_{s\,opt}$, is available, the straightforward way to guarantee that the process is kept on that path is on-line control. Here we use a direct model predictive control technique to illustrate the capabilities of HYBNET. Model predictive control directly makes use of the explicit and separately identified hybrid process model in order to indirectly determine the biomass from the available measurement data, it also makes use of the measurement values, e.g., the fermenter weight, in order to determine a reliable value of the culture volume v. Together with the substrate concentration s_F in the feed, the actually required feed rate F_{opt} can be determined by

$$F_{opt} = q_{s\,opt} \text{ x } v/s_F \tag{2}$$

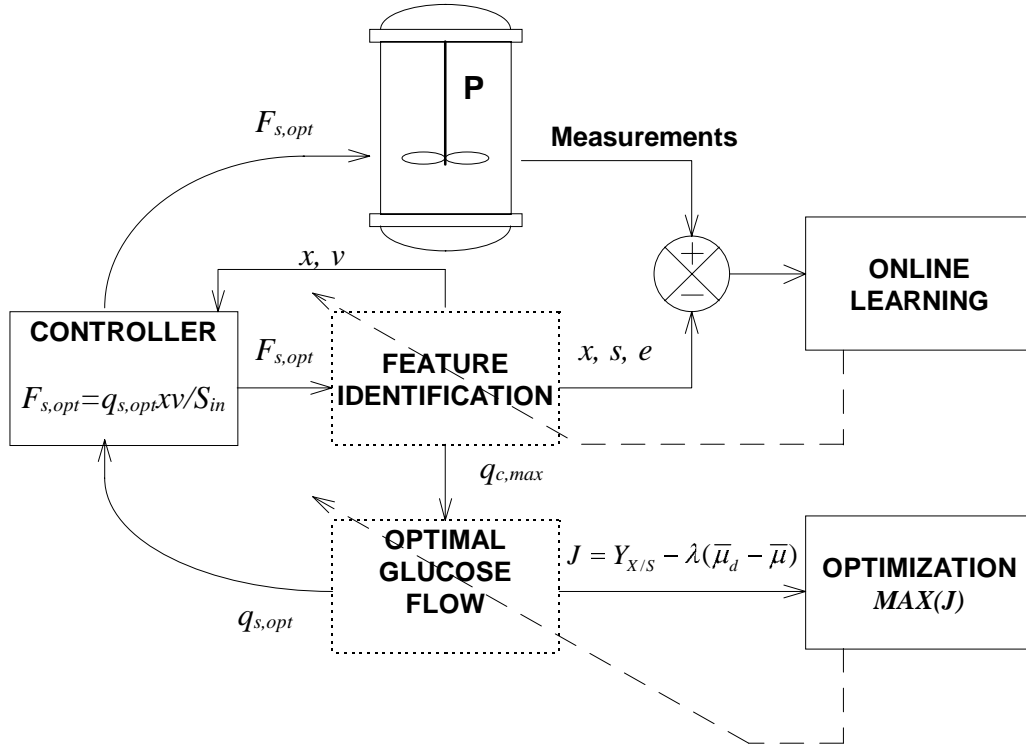This signal can be transferred directly to the physical feeding system.

Fig. 7. Hybrid network for online optimization. The feature identification and optimal glucose flow modules are condensed representations of the diagrams in figs 3 and 5 respectively.

In order to further improve the control and to respond on modeling and measurement errors, the predetermined feature profile can be corrected on-line. Such tuning can be done with HYBNET in the way sketched in Fig. 7. The currently available measurement are compared with the model predictions and as soon as there is a significant deviation,

the feature $q_{c\ max}$ will be corrected in a error feed-back fashion. Any significant change then also requires an adapttion of the $q_{s,opt}$ profile. With the new data of $q_{s\ opt}$, the set point for the F_{opt} valve is then corrected.
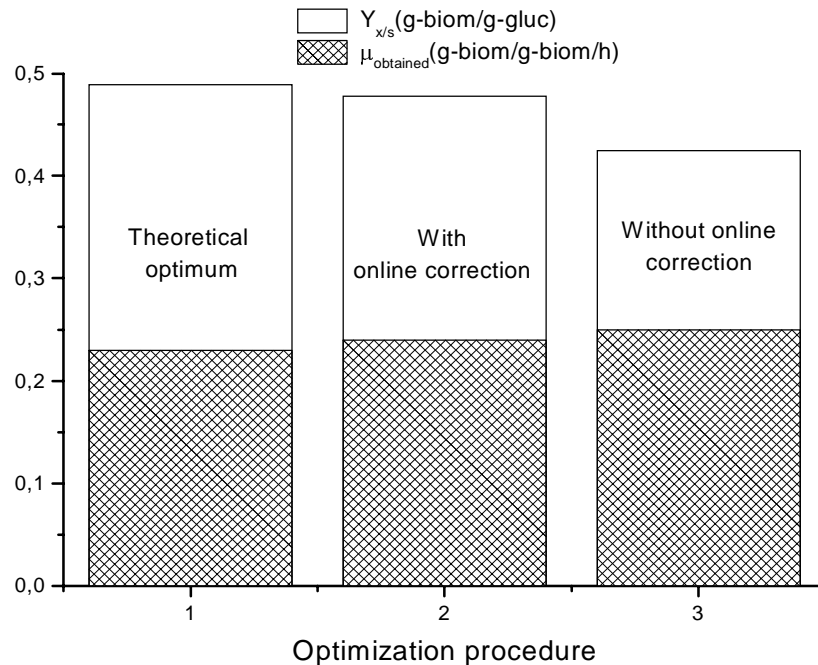

Fig. 8. Comparison of the optimization procedures with a test fermentation: procedure 1) Theoretical optimum Yxs=0.49 $\mu_{obtained}$=0.23; procedure 2) online implementation with correction Yxs=0.48 $\mu_{obtained}$=0.24, and procedure 3) online implementation without correction Yxs=0.42 $\mu_{obtained}$=0.25.

The decisive advantage of this procedure (Oliveira et al. 1997) in comparison to the classical direct optimization of the feed rate profile $F(t)$ is that the focus of the control is on the physiological key control quantity, the specific substrate consumption profile and the potential errors in assuming biomass and volume profiles (which are necessary to determine $F(t)$) are significantly reduced (viz Fig. 8). E.g., there are particularly problems in determining the $v(t)$ profile in laboratory experiments and pilot plants where base addition, evaporation, feeding of antifoam agents etc. can lead to significant errors in the prediction of $v(t)$. Also errors in the concentration s_F can lead to significant errors in practice. With state estimation procedures using actual measurement data during the running process, the profiles of x and v can be determined with a considerably smaller error.

5 CONCLUSIONS

The widespread use of the results of advanced methods of process optimization and control is largely delayed by missing software-tools that can help to keep the

expenditures for development and maintenance in acceptable limits. HYBNET is a development done in order to cope with that problem.

HYBNET helps to reduce as much as possible the formal efforts to develop a process model that can be used for process optimization and control. It provides the corresponding guidance for the biochemical engineer. However, it is not possible what some vendors of software packages claim that modeling of complex biochemical processes is an easy task. The biochemical engineer must perform hard work to collect all the currently available knowledge which might be relevant to his particular task. It is also not correct that all problems can now be solved merely with artificial networks or fuzzy experts systems alone. For instance, mechanistic descriptions already available must be used directly, it does not make sense to learn them once more from noisy data. HYBNET will help him to decide whether or not it will have a significant impact on the process benefit/cost-ratio and in case of a positive decision to integrate it into the network constituting the current process model.

In HYBNET, however, the process model is merely considered a means to an end. The focus is on the task to be performed, e.g., the optimization of the productivity. Thus, the afford to construct a model must carefully rated with an eye on the benefit/cost-ratio. A central issue in HYBNET is process optimization, e.g., the determination of optimal feeding profiles in fed-batch processes as well as the start parameters like start volume, start substrate concentration. Several ways are provided to support such tasks depending on the knowledge that can be made available. In the beginning, when there are only a few data available the weight is more on knowledge from literature. When there is more data available the focus moves more and more to data driven techniques, since they more directly reflect the behavior of the particular process under consideration. The idea of fusion of models is not new, but HYBNET is a tool which allows to bring it into practice with a reasonable expenditure.

HYBNET was developed to support advanced control strategies in industrial production environments. There process control is one major issue. This problem has been solved in very much the same way than process modeling. The process is then viewed at with the controller being an integrated part of the process. Consequently, the hybrid techniques developed, could be simply extended. And, the determination of the controller parameters become an optimization problem, where the process performance is used as the optimization criterion.

HYBNET is used in different biochemical production processes as well as in laboratories and pilot plants. It is continuously being extended in particular concerning the user interface, which is necessary to reduce the activation barrier felt by many process engineers in industry for using software tools.

REFERENCES

Oliveira, R., G. Smolders, R. Simutis, A. Lübbert (1997). Improved on-line control of biochemical cultivation processes. (in preparation)

Rumelhart, D., D. Hinton, G. Williams (1986). Learning Internal Representations by error propagation. in D. Rumelhart and F. McClelland eds., *Parallel Distributed Processing,* **1,** Cambridge, MA:M.I.T Press

Schubert, J., R. Simutis, M. Dors, I. Havlik, A. Lübbert (1994). Bioprocess optimization and control: Application of hybrid modelling. *J. Biotechnol.* **35**, pp. 51-68

Simutis, R., A. Lübbert (1997). A comparative study on random search algorithms for biotechnical process optimization. *J. Biotchnol.*, **52**, pp. 245-256

Simutis, R., R. Oliveira, A. Lübbert (1996). Hybrid Modeling with Neural Networks and its Utilization in Bioprocess Control. Bioreactor Engineering Course, Saltsjöbaden, June 14-18

Sonnleitner, B., O. Käppeli (1986). Growth of *Saccharomyces cerevisiae* Is Controlled by Its Limited Respiratory Capacity: Formulation and Verification of a Hypothesis. *Biotech. Bieng.*, **28**, öp. 927-937

Sulzbach, D. (1997). An industrial perspective on scientific computing, Http://www.stewart.cs.sdsu.edu

Werbos, J. P. (1990). Backpropagation Through Time: What It Does and How to Do It. *Proceedings of the IEEE,* **78(10),** pp.1550

Chapter 4

Closed-loop Control Using an On-line Learning Hybrid Model Network

Abstract. This paper presents a model-based closed-loop control procedure based on a hybrid process model. An inferential control strategy not yet discussed in literature, was chosen to keep the concentrations of ammonia and precursor in penicillin production experiments under tight control. Its main component, the estimator for the ammonia and precursor consumption rates, is an indirect measurement procedure which uses several different on- and off-line measurement data. An hybrid process model was used to combine several kinetic models, which was capable of learning during its application using automatic training techniques. Particularly, the neural network component of the hybrid network was retrained during this on-line learning process. Also all the other components of the hybrid model are automatically re-tuned, once new data becomes available. The proposed procedure was tested in 22 fermentation runs where it proved to be robust and stable.

1 INTRODUCTION

Improvement of the benefit/cost-ratio of their fermentation processes is one of the most important every-day-duties of biochemical engineers. For cost reasons, typically only a few experiments can be conducted, particularly when fermentation processes for highly competitive products must be improved. Strain selection or control profile optimisation are typical examples of the concrete aims. Hence, the necessary experiments must be well designed and tightly controlled in order to provide sufficiently accurate information on which the relevant decisions may be grounded. Obviously, data from badly controlled experiments, which depict a batch-to-batch variability of several percent cannot be used to distinguish between the benefits or disadvantages of different control profiles or strains with respect to the process performance, when the changes are expected to be in the same order of magnitude.

In the case of strain selection experiments, where the influence of different strains on productivity is tested, process control is of paramount importance, since it must be guaranteed that the different strains are compared on exactly the same base, i.e. under well defined conditions.

In order to control a fermentation process, a considerable amount of knowledge must be activated. Several approaches have been discussed in literature. However, the problem with many investigations on control published in the biotechnological literature is that they are theoretically brilliant studies but the results most often were not validated within a sufficiently large number of fermentations. This paper is intended to describe a model-supported closed-loop control procedure that already proved to work in practice.

The procedure is explained at the example of the penicillin production process, which is one of the standard processes with respect to process control discussed in literature over many years (Constantinides *et al.,* 1970; Bajpai and Reuss, 1981; Nielsen and Villadsen, 1994; DiMassimo *et al.* 1992; Preusting *et al.*, 1996). Most often, open loop control was discussed in literature. However, the main disadvantage of an open-loop control is that uncertainties in the initial conditions and system parameters may lead to large errors in process operation. Therefore, it is advantageous, to develop a closed-loop optimisation scheme which attenuates uncertainties in the parameters and is fairly independent of the initial conditions (e.g., Modak and Lim, 1987).

Here we address a particular control problem in the penicillin production process that has not been extensively discussed in literature, the regulation of the concentrations of the precursor and the nitrogen source. The addition of side chain precursor stimulates the synthesis of penicillin (Thorn and Johnson 1950), but it can be toxic at too low pH values. Ammonium salts are suitable sources of nitrogen, however, the concentration of free NH_4^+ should be kept constant on an appropriate level, since this may influence the synthesis of certain amino acids needed for penicillin synthesis (Court and Pirt 1981, Lurie *et al.* 1976).

It was concluded from previously performed studies that the precursor and ammonia concentrations should be kept at constant levels. Deviations from their quasi optimal values were recognized as to decrease the penicillin productivity. Hence, it is straightforward to regulate these concentrations, C_n, C_{pa}, so that they are kept within well defined bounds around their set points $C_{n,sp}$ and $C_{pa,sp}$.

Today, such a control of precursor and the ammonia concentrations is being performed manually in most factories. But this way of controlling is expensive, since it binds considerable man-power, requires an extensive personnel training and the quality of control depends on the personal skills of the operator. The less expensive alternative is computer based process supervision and control.

What type of control is necessary? Since we are generally forced to look for the most simple solution, the first question that must be asked is whether or not the simple PID controller can be applied. There are two reasons that speak against this simple solution.

The most severe practical problem is that the control variables, C_n, C_{pa} are usually measured off-line with a long sampling time increment Δt, which is much larger than can be accepted for direct control. Hence, there is no measurement signal that can be used in the PID controller.

The second reason is that PID controllers react on deviations in the actual value of the control variable from its corresponding set point in a predefined way specified by the controller parameters. The parameters of simple PID controllers are adjusted to the nominal process dynamics, which is assumed to be time-independent and known beforehand. The penicillin production process, however, is known to be a strongly nonlinear process that depict a substantially time varying dynamics.

Hence, alternative controller designs, which can cope with these two problems, must be considered.

In this paper we propose to use the inferential control technique, which emanated from model predictive control techniques (Garcia *et al.,* 1989). In inferential controllers, the information about the process state is determined indirectly by means of a model supported measurement. Hence, the direct measurement of the control variable, necessary in a simple controller, is replaced by using other measurement information and a sufficiently accurate relationship between the data and the control variables. In this way it is possible to cope with the long time increments Δt mentioned before.

When, the relationships connecting the measured variables with the control variables are dynamic relationships, the problem concerning the time-varying process dynamics is also solved. Hence, inferential controllers are the matter of choice for the problem to be solved.

The measurement variables that can be used in the indirect measurements of the ammonia and precursor concentration are: substrate feed rate F_s, ammonia feed rate F_n,

precursor feed rate F_{pa}, fermenter weight W_B, carbon dioxide production rate CPR, and oxygen uptake rate OUR.

In an inferencial control system, the accuracy of the supporting estimation model is of major importance. For the present problem, accurate models that relate the control variables with the manipulateble quantities are not available in literature. Hence, they had to be developed. Accuracy increases with the amount of relevant a priori knowledge about the process that can be activated to aid the control task. For that case it is straightforward to make use of hybrid models as they are a mean to take the available knowledge or information about the relationships from different sources as they become available, thus avoiding losses which appear during any transformation into any other representation. In such a hybrid model we make use of model structures available in literature as well as of heuristics and the information still hidden in data records measured at the process.

Since in the beginning of the construction of a model, the knowledge which can be activated is limited, it is straightforward to adapt the model to the process under consideration whenever new data, or any other kind of new information about the process behavior, becomes available. In this paper we use an on-line learning strategy.

2 PROCESS MODEL

Since the process model is of decisive importance in an inferential controller, the process model used for this purpose is separately discussed in detail.

As the process model is used here to aid the inferential controller, its main objective is to provide a sufficiently accurate link between the control variables and the measured quantities in such a way that the process controller can keep the ammonia and the precursor concentrations within predefined intervals of $\pm E_n$ and of $\pm E_{pa}$ around the respective setpoint values.

The demands for these error bounds were derived from the reproducibility requirements of the production process. They were determined in previous investigations and are considered to be concretely given in the work reported about here.

2.1 Macroscopic Balances

The backbone of the hybrid model discussed in this paper is a system of mass balance equations for the central quantities in cause. In the penicillin process, there are 7 quantities that play a decisive role. These are the concentrations of biomass, substrate, product, precursor, nitrogen source, oxygen and carbon dioxide. It would be optimal if one could take into account all these relevant variables. In practice, however, the first three quantities cannot be measured on-line with sufficient reliability. Even an indirect measurement of these variables can be performed with considerable errors only. The latter two quantities can be covered by on-line measurements. Hence, we must deal primarily with the two control variables C_n and C_{pa}. Then, of course, it must be shown

that this choice suffices to cope with the control problem stated above. Test calculations showed that the incorporation of the balances for biomass, substrate and penicillin did not lead to advantages with respect to the estimation of C_n and C_{pa}. The total mass of the system is known from on-line measurements and, therefore, does not need to be considered in the balance equations.

For the fed-batch regime, usually applied in the penicillin production, the mass balances across the culture within the fermenter can be represented by the following simple system of ordinary differential equations

$$\frac{dC_n}{dt} = -R_n + \frac{F_n}{W_B} C_{n,fn} - \frac{F_{tot}}{W_B} C_n \tag{1a}$$

$$\frac{dC_{pa}}{dt} = -R_{pa} + \frac{F_{pa}}{W_B} C_{pa,fpa} - \frac{F_{tot}}{W_B} C_{pa} \tag{1b}$$

where the vector C_i represents the concentrations of precursor and ammonia and C_{fi}. the corresponding concentrations in the feed into the reactor. F_{tot} is the total rate of volume change within the system boundaries, which besides the feed rates may also contain contributions of water evaporation and losses due to sampling events. When the feed rates F_i are known, the only components that deserve special attention are the rate expressions R_n and R_{pa}. These rates are determined by the process kinetics.

2.2 Kinetics

The kinetic model must be derived from the available data of a set of experiments. Hence, it is straightforward to first analyze this data in a conventional way (DiMassimo et al. 1992).

An immediate question appearing in this respect is the question about the variance in the data of a set of experiments performed under comparable conditions. In order to estimate the variance, the average consumption rate profile was extracted from a set of 32 fermentation data records. The ensemble averaged results are shown in Figures 1 and 2 together with the original data.

In order to judge the variance in the data, i.e. the deviations of the measured rate profiles (obtained in the individual fermentation) from these average profiles, and the intervals corresponding to the error bounds allowed, are determined. These error bounds for the rate data can be estimated from the above mentioned concentration error bounds by the following simple approximation

$$E_r = E_c/\Delta t \tag{2}$$

where Δt is the time increment of the data sampling procedure. The same approach was used for both control variables. The corresponding intervals around the ensemble averages are also depicted in the Figures 1 and 2. These two Figures show that both data clouds do not stay within the predefined error bounds. Hence, we can conclude, that

both rates must be estimated more accurately. The ensemble average can be regarded a first guess for the rate profile of a representative fermentation.
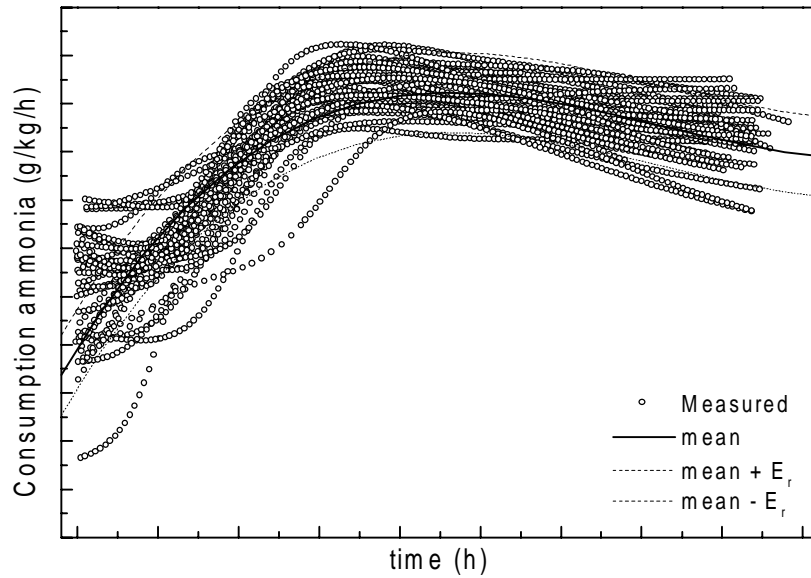

Fig. 1. Consumption rates of ammonia for 32 fermentations
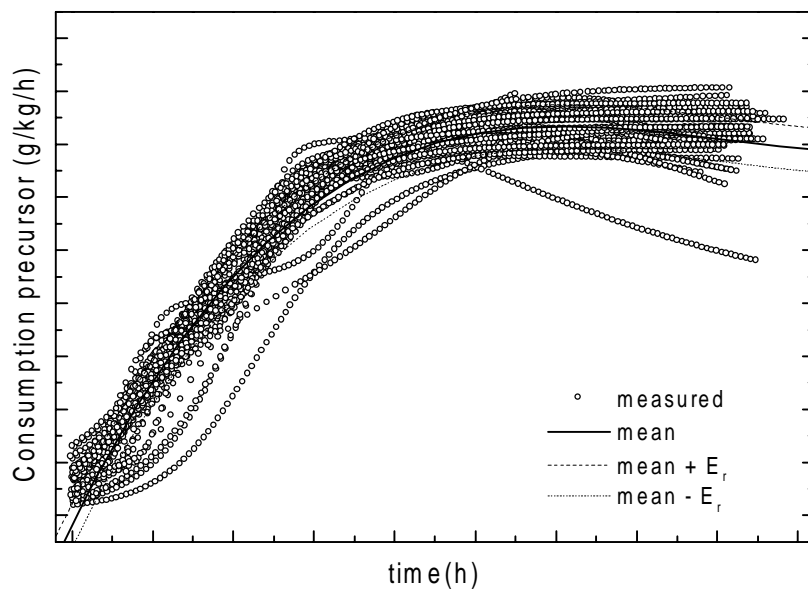

Fig. 2. Consumption rates of precursor for 32 fermentations

To construct an accurate kinetic model, it is necessary to know the interrelationships between the measured process variables and the rate expressions. Thus, the appropriate next step in the statistical analysis should be a correlation analysis. The results of such a correlation analysis are summarized in Tables I and II. They are provided in the form of correlation matrices of the variables t, R_s, CPR, RQ and R_n and the variables t, R_s, CPR, RQ and R_{pa} respectively. For both, the ammonia and precursor consumption rates, it can be seen that the highest correlation is with the glucose consumption rate R_s, followed by CPR, t, and finally RQ. In the case of precursor, the correlation with t is notably high, what means that the precursor signal has a prominent structure or time pattern. The result of the correlation analysis can be regarded a ranking of the variables. This must be considered in a detailed kinetic model.

Table I – Correlation matrix for the set of variables t, Rs, CPR, RQ and Rn

	t	R_s	CPR	RQ	R_n
t	1	0.7685	0.8795	0.3368	0.5787
R_s	0.7685	1	0.9029	0.2895	0.8696
CPR	0.8751	0.9029	1	0.3098	0.7752
RQ	0.3368	0.2895	0.3098	1	0.1889
R_n	**0.5787**	**0.8696**	**0.775 2**	**0.1889**	**1**

Table II – Correlation matrix for the set of variables t, Rs, CPR, RQ and Rpa

	t	R_s	CPR	RQ	R_{pa}
t	1	0.7685	0.8795	0.3368	0.8300
R_s	0.7685	1	0.9029	0.2895	0.9388
CPR	0.8751	0.9029	1	0.3098	0.9045
RQ	0.3368	0.2895	0.3098	1	0.2936
R_{pa}	**0.8300**	**0.9388**	**0.9045**	**0.2936**	**1**

When, in a first approach, a linear relationship between the ammonia and precursor consumption rate and the substrate consumption rate R_s, which appeared as the first candidate of an influence variable in the correlation analysis, is assumed then, for 32 fermentation data sets, we obtain the rate profiles depicted in the Figures 3 and 4. Although these representations show that there is some linear component in the data, the linear representation is not satisfactory. In particular the dependency of the precursor rate from the substrate consumption rate deviates too much from the allowed error bounds indicated in Figure 4.
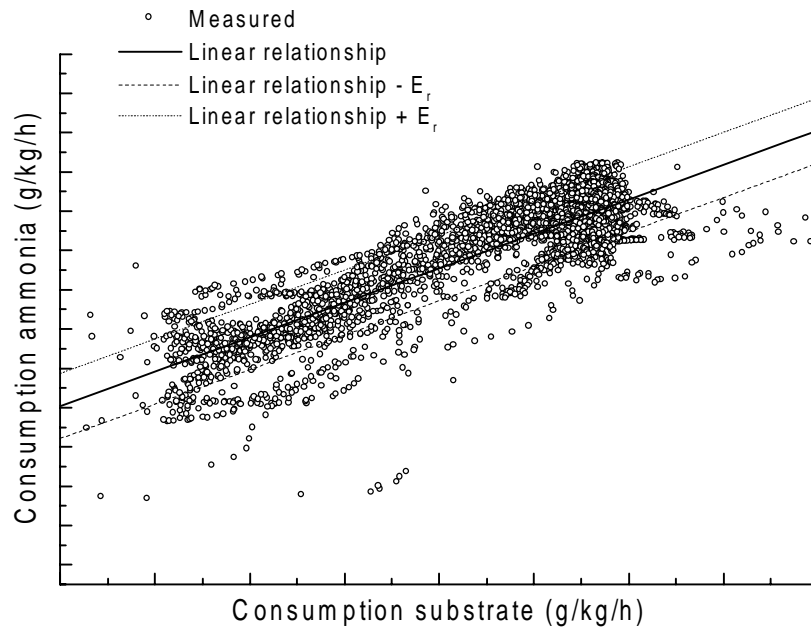
Fig. 3. Linear relationship between the consumption rate of ammonia and the consumption rate of substrate
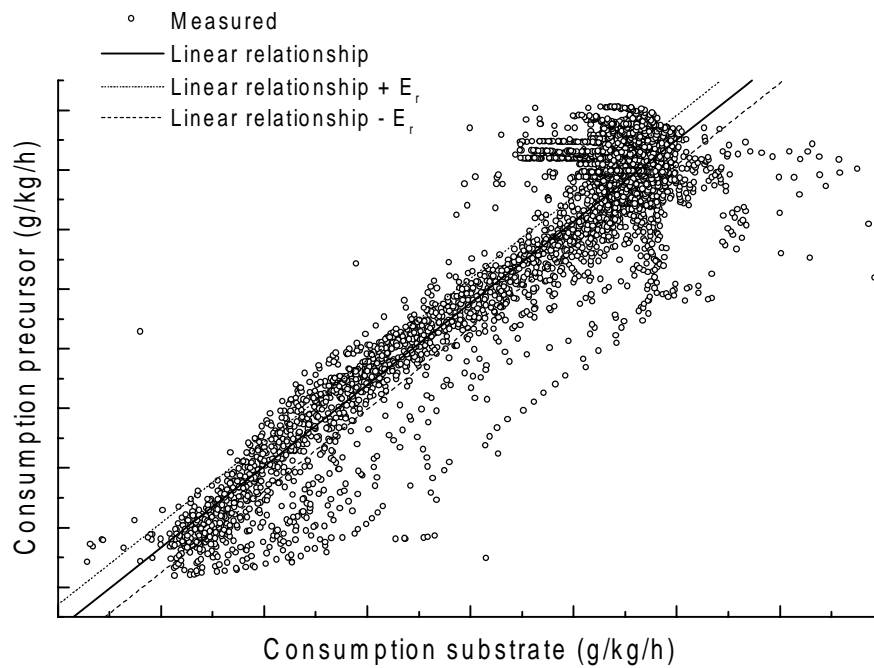

Fig. 4. Linear relationship between the consumption rate of precursor and the consumption rate of substrate

The standard deviation of the precursor consumption rates from its mean is about 1.8 times the value that, following the above mentioned error estimation, was considered to be allowed. Hence, a more complex model in particular for the precursor, but also for the ammonia consumption rate is needed.

2.3 Hybrid Model Network

Since in this particular application a considerably extended set of fermentation records is available, it is straightforward to use a data driven approach to develop an accurate kinetic model that considers more than a single influence variable. Here we propose to use an hybrid model network to describe the process kinetics. As has already been recognized by several authors (Psichogios and Ungar, 1992; Thomson and Kramer, 1994; Schubert *et al.*, 1994) such hybrid models can be exceptionally powerful in representing nonlinear process kinetics. In the hybrid kinetic model proposed in this section, several blocks that simultaneously describe the same phenomenon, are used in the sense of providing different votes for the rate expressions needed, emanating from different points of view. In order to combine these votes properly, there is an arbiter that decides about the relative weights which must be attributed to the different contributions.

The motivation for this approach is that the relevant knowledge is available by part in form of mechanistic models, by heuristic rules, and by part simply in form of data structure information, which is most effectively represented by artificial neural networks. Since any attempt to transform all this knowledge into a single representation, for example into a mathematical model means a considerable loss in information, the different items are represented in their most direct form. This is the mathematical equation for the mechanistic model, a fuzzy rule system for the heuristics as well as a neural network to represent the structural information from extended data sets.

2.3.1 Neural network module. In the beginning of such a model development, when mechanistic knowledge is not so well developed, the neural network component takes the main load of an hybrid model, particularly from the point-of-view that it can be regarded as an advanced nonlinear correlation technique. In this work a conventional feedforward neural network was used with three layers to represent the two rate expressions R_n and R_{pa} considered in our kinetics. As input variables, the 4 quantities that were found to correlate best with the rate expressions were taken: Substrate feed rate F_s, time t, carbon dioxide production rate CPR, and respiration quotient RQ.

2.3.2 Fixed carbon module. The second approach to represent the kinetics is the so-called fixed-carbon model. It is based on the heuristic experience that the rates considered do correlate better with the carbon fixed in the bioreaction system than with the substrate consumption rate. The amount f_c of "fixed carbon" is determined from the flow of carbon entering the system by means of the substrate feed and the flow of

carbon leaving the system via the CO_2 through the vent line. This can be approximated by

$$f_c = \frac{R_s}{M_s} - CPR \qquad (3a)$$

where (R_s) is the substrate consumption rate, (M_s) is the molecular weight of substratct in C-mol. The rate by which nitrogen is consumed is assumed to be linearly dependent on the carbon consumption rate

$$R_{n,fc} = a_1 \, f_c + b_1 \qquad (3b)$$

For the precursor consumption a similar approach was followed, however, it proved to be necessary to assumed time-dependent coefficients.

$$R_{pa,fc} = a_2(t) \, f_c + b_2 \, (t) \qquad (3c)$$

The coefficients a_i are in some sense yield coefficients. The absolute terms b_i can be interpreted as some part of the maintenance requirements.

2.3.3 Representative Trajectory. The third representation of the relevant rates is a set of representative time profiles for the two rates $R_n(t)$ and $R_{pa}(t)$ which represent a typical well performing cultivation. These profiles were constructed from the experience with the fermentation system.

It is of advantage to comprise this profile in a some more convenient representation. Since these profiles are rather complex, these time functions may be represented by means of feedforward artificial neural networks. This allows to works with several different fermentation profiles that can be used later on for different cases. The operator can decide afterwards which of the representative profiles fits best with the actual fermentation.

2.3.4 Weighting the modules. The weights by which the different modules are taken into account during the model evaluation are determined with a small expert system. The main emphasis was placed on the experimental evidence available in the corresponding part of the state space. The algorithm used to determine this evidence is the so-called evidence measure (Leonard and Kramer, 1992). This evidence measure characterizes the credibility of the models that used the corresponding measurement information.

Most priority is attributed to the neural network description of the kinetics, since it proved to provide the most reliable results at regions in the state-space where the evidence measure is high. The extrapolation measure is a number between 0 and 1. This number was taken to determine the relative influence of the ANN on the rate expression. The complementary weight was distributed on the two other models. In cases where there are measurement errors in the CPR, the fixed carbon model cannot be used. Then, the representative fermentation profiles are given the preference. Hence, the

relative weight of the other two models was made dependent of the accuracy by which the CPR could be measured. Only a few rules suffice to set up the small fuzzy expert system that chooses the relative weights.

2.4 Identification

The model identification, i.e. the fit of the model parameters to the available data sets, can essentially be considered an optimization problem. The expenditure required for such an identification depends on the complexity of the model used. With hybrid model networks, we are faced with rather complex models where simple optimization procedures might not work properly. In the case of neural networks the identification is referred to as the network training.

The most used training methods for neural networks are based on the error backpropagation technique, which enables the exact calculation of gradients of a given objective function with respect to the neural network parameters (Werbos, 1990). However, in the case of hybrid model networks that contain further different model components, and in particular when they include differential equations, error backpropagation must be used together with the sensitivity technique in order to calculate the required gradients (e.g. Schubert *et al.,* 1994; Oliveira *et al.*, 1998). In the present work the learning method was based on the following techniques:

1. Batch least-squares objective function
2. Error-backpropagation+sensitivities methods to calculate the objective function Gradients with respect to the hybrid model network parameters
3. Conjugate gradients with line-search optimization algorithm
4. Cross-validation validation technique

The objective function consisted on a minimization of precursor and ammonia estimation errors according to a least-squares criterion:

$$E = \left(\frac{1}{P} \sum_{t=1}^{P} ([\ \alpha(C_{pa,e}(t) - C_{pa}(t))\ ]^2 + [\ \beta(C_{n,e}(t) - C_n(t))\ ]^2) \right)^{\frac{1}{2}} \qquad (4)$$

where $C_{pa,e}$ and C_{pa} are the measured and estimated concentration of precursor, $C_{n,e}$ and C_n are the measured and estimated concentrations of ammonia, α and β are scaling factors derived from the variances of the corresponding variables, and t a set of P measured values of the ammonia and precursor concentrations.

The objective function gradients are obtained by differentiating eqn. (4) with respect to the hybrid model parameters vector W

$$\frac{\partial E}{\partial W} = -\frac{1}{E\ P} \sum_{t=1}^{P} \left(\alpha(C_{pa,e}(t) - C_{pa}(t)) \frac{\partial C_{pa}(t)}{\partial W} + \beta(C_{n,e}(t) - C_n(t)) \frac{\partial C_n(t)}{\partial W} \right) \qquad (5)$$

The computation of vectors $\partial C_{pa}/\partial W$ and $\partial C_n/\partial W$ in eqn. (5) can be done with the sensitivities method and error backpropagation in the hybrid model network (Oliveira *et. al*, 1998).

The optimization consisted on a conjugate gradients with line-search algorithm employing the gradients estimates given by eqn. (5). This strategy proved to be most efficient for training artificial neural networks (Leonard and Kramer, 1990) and hybrid model networks (Oliveira *et. al*, 1998).

Process models can only be used in industrial practice after they have been carefully validated (DiMassimo et al. 1992). In the present work this was performed by means of a cross-validation procedure (e.g. Polard *et al.*, 1992) where some part of the experimental data available, which has not been used during the identification procedure, is used to test the model. 70% of the available data was used for process model identification, while 30% was used for validation.

The errors that appeared in the validation tests were close to that needed for the control problem stated above. But unfortunately only in 71 % of all runs considered the errors were small enough. This means that the initially developed model does not perform well enough. Hence, on-line correction strategies should be adopted.

3 CONTROLLER DESIGN

The controller algorithm is obtained from a direct application of the process model. The controller actions are dependent from the deviations from the set points $C_{n,sp}$ and $C_{pa,sp}$ and the estimates C_n and C_{pa} for the ammonia and precursor concentrations. When there are strong changes in the deviations, the usually applied controllers act dramatically. Jumps in the deviations between set points and the estimated concentrations are to be expected in our particular case whenever we obtain new off-line measurement data for ammonia and precursor, since they are used to correct the actual estimation.

In order to avoid overreactions, the controller actions were constrained so that the set point profile is approached smoothly but as quick as possible. Thus, the controller was designed to act with a first order dynamics in the following way

$$\frac{dC_n}{dt} = - \frac{C_n - C_{n,sp}}{\tau} \tag{6a}$$

$$\frac{dC_{pa}}{dt} = - \frac{C_{pa} - C_{pa,sp}}{\tau} \tag{6b}$$

The time constant τ is determined from the operator experience. Combining these equations with the model (eqns.1) leads to the following equations for the manipulateble quantities:

$$F_{pa,rec} = \frac{(C_{pa} - C_{pa,sp})W_B}{\tau \, C_{pa,fpa}} + \frac{R_{pa}W_B}{C_{pa,fpa}} \tag{7a}$$

$$F_{n,rec} = \frac{(C_n - C_{n,sp})W_B}{\tau \, C_{n,fn}} + \frac{R_n W_B}{C_{n,fn}} \qquad (7b)$$

The first terms in the right hand sides of these equations correspond to the feedback component of the controller, the second terms to the feedforward part. Fig. 5 provides a schematical view of this feedforward/feedback control structure.

Notice that to solve eqns. (7) the values of C_{pa}, C_n as well as the ones of R_{pa} and R_n are required. These values are provided on-line by the process hybrid model, which works, as mentioned before, as an indirect measurement system.
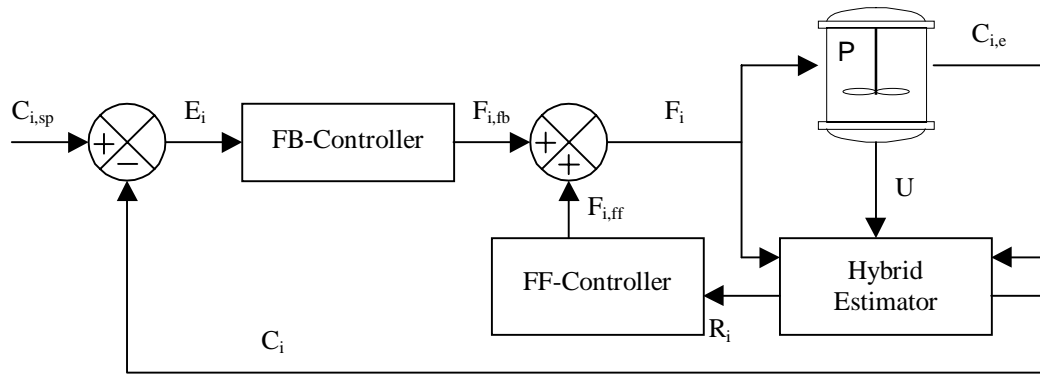
Fig. 5. Feedback/feedforward closed-loop control structure (i=ammonia, precursor)

4 ON-LINE LEARNING PROCEDURE

By on-line learning we understand an improvement of the kinetic model during the application of the system by using the data becoming available with the running fermentation. In this way it is possible to adapt the process model used in the model-supported closed-loop. Two aspects must be distinguished in this respect. The first is that with more data, the database for training the models becomes broader and hence the models become more accurate in general. The second is that by using the data from the particular running process, the model can be adapted more closely to the actual process behaviour.

The kinetics are described with an hybrid model which contains global information in form of the control profiles for a representative cultivation as well as specific information represented by neural nets. The incentive here is to update the neural network components by means of an additional training using the currently incoming data.

The main idea behind the on-line training procedure is to use a *Dynamic Reference Database* which primarily contains a set of historical data records representing fermentations performed in the past that were classified good examples. This data base is complemented with the measurement information from the running fermentation. Each time a new off-line value of precursor or ammonia concentration becomes

available, the training procedure is activated on the data contained in the reference data base.

In principal, the same training procedures as with the off-line training can be used. However, one must take care that the neural network does not forget what it already learned before. Thus an appropriate relative weighting of the actual data and the already incorporated information must be performed.

The steps concretely performed are the following:

1. The training is performed with the data in the *Dynamic Reference Database*, containing data from about 5 reference fermentations, and the data of the running fermentation.
2. The maximum step size for the line-search algorithm is fixed to a low value (c.a. 0.1) to avoid that the hybrid network forgets what it learned in the past.
3. The maximum number of iterations was set to 100 only for the same reason.
4. The 3 parameters, i) the number of historical fermentation data records in the *Dynamic Reference Database*, ii) the step size for the line-search algorithm and iii) the maximum number of iterations were chosen heuristically. Figure 6 schematically depicts the structure of the closed-loop controller applied including the on-line learning strategy.

For cases where the additional on-line training does not lead to an improvement in the model accuracy and hence in the controller performance, the relative weight of the data from the actual fermentation is increased so that the controller is more directly adapted to the currently running process. The decision is made by another small expert system incorporated in the software.
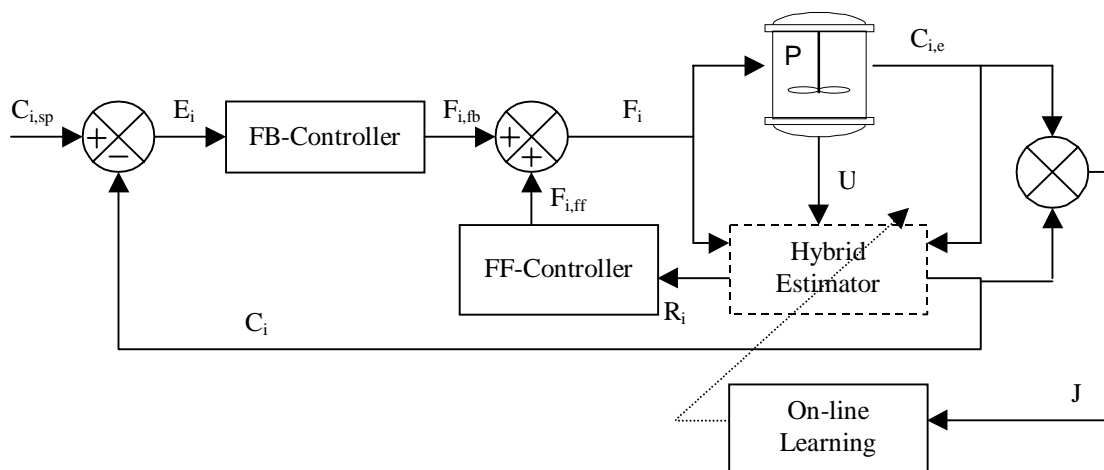

Fig. 6. Feedback/feedforward closed-loop control system with on-line learning (i=ammonia, percursor)

The practical consequence of the model improvement is that once the basic model changes, all what has been derived from that model is also adapted. This is particularly the set of feeding profiles for precursor and ammonia. Thus, whenever the model was changed the profiles are corrected, and by proceeding in this way, the robustness of the control system is considerably improved.

5 IMPLEMENTATION

The inferential controller was implemented at a fermentation system on laboratory scale which was equiped with a front-end computer process monitoring and low-level control. The inferential controller was implemented on a workstation which was linked to the front-end computer.

A Digital Equipment AlphaStation was used for supervision and advanced control. It runs under the Digital UNIX operative system. This workstation is coupled to the PC-based front-end computer system placed in the fermentation hall via a standard TCP/IP network.

Hybrid model formulation, hybrid model identification, and on-line control, were performed with the HYBNET software package (Oliveira *et al.,* 1998). This package was installed in the AlphaStation running under the Digital UNIX operative system. The communication protocol was built on a base of file transfer (FTP-protocol), in which the process data matrix is continuously sent to HYBNET and the set-point table was continuously sent the other way around to the front-end PC.

The set of software tools and HYBNET configuration files implementing the control system was named "BUBE system". BUBE is formed by all the HYBNET configuration files implementing i) the process hybrid model, ii) the controllers, and iii) the communication protocol. It includes additionally a few software tools specially developed to fit the needs of the people working daily with BUBE. In particular, a personalized graphical interface was developed to improve the user-friendliness and the visualization capabilities of the system.

6 DISCUSSION AND CONCLUSIONS

The software developed was used to control a series of penicillin fermentations in the laboratory. After 17 fermentations controlled with BUBE it is possible to compare the results of a statistical base with an arbitrarily chosen set of manually performed fermentations. For comparison a set of 12 manually controlled fermentations was taken. The first information of interest is the mean deviation of the control variables from their corresponding set points as averaged over both sets of fermentations. A comparison of the fermentation results is depicted in Figures 7 and 8 for the case of ammonia. The same procedure can be performed for the precursor concentration. The corresponding data are shown in Figures 9 and 10.
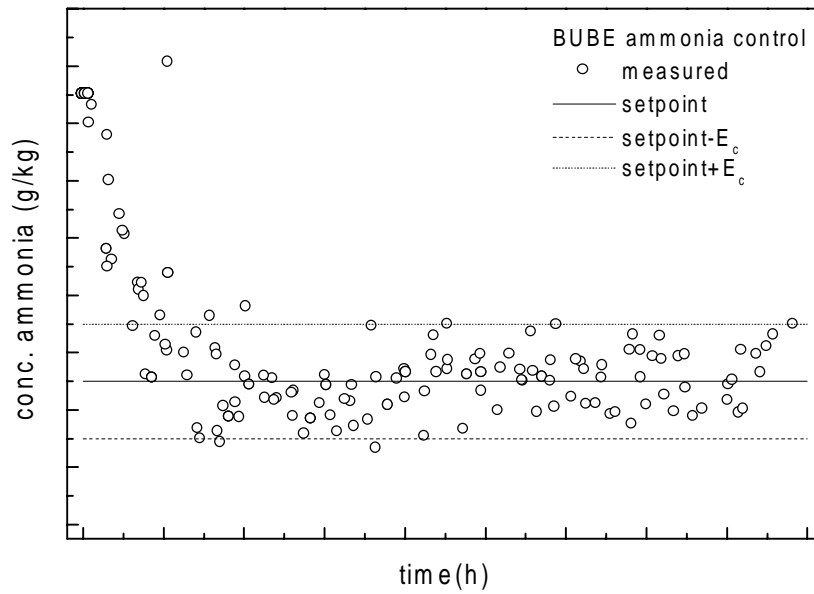
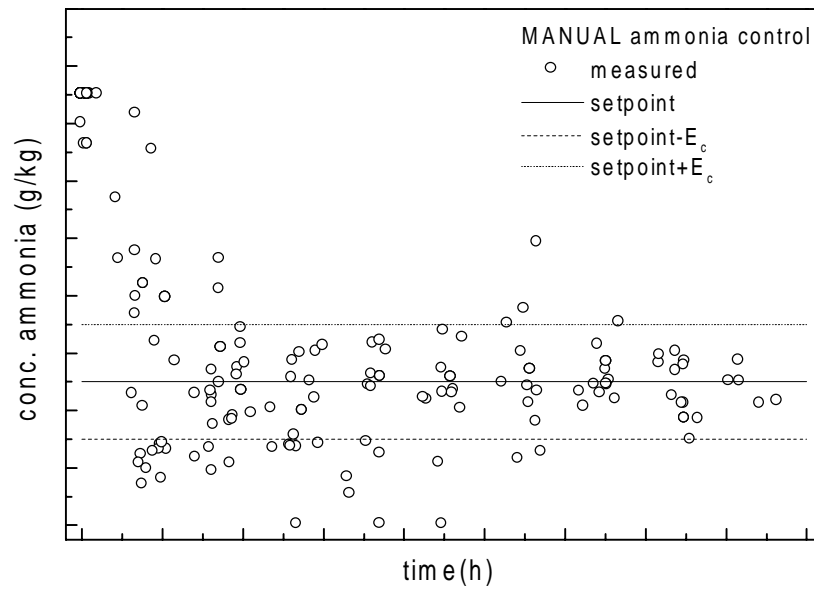Fig. 7. Concentration of ammonia for 17 fermentations controlled by BUBE

Fig. 8. Concentration of ammonia for 12 fermentations controlled manually
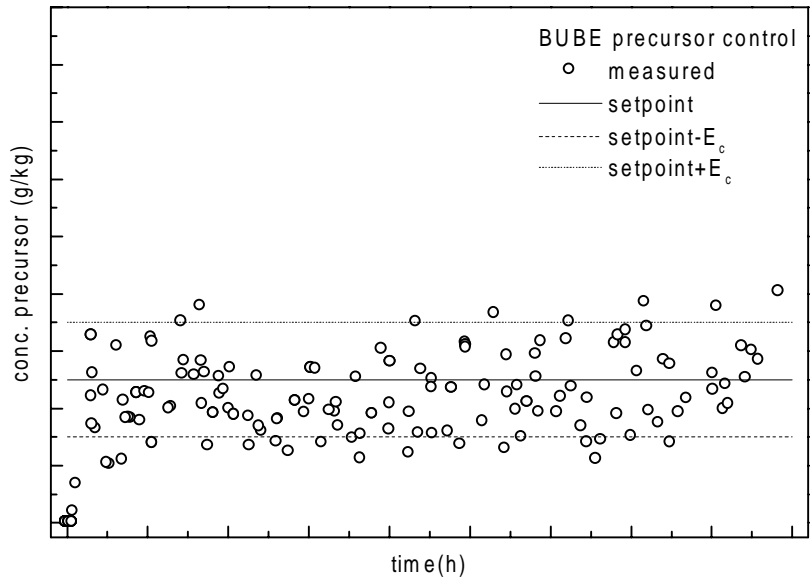
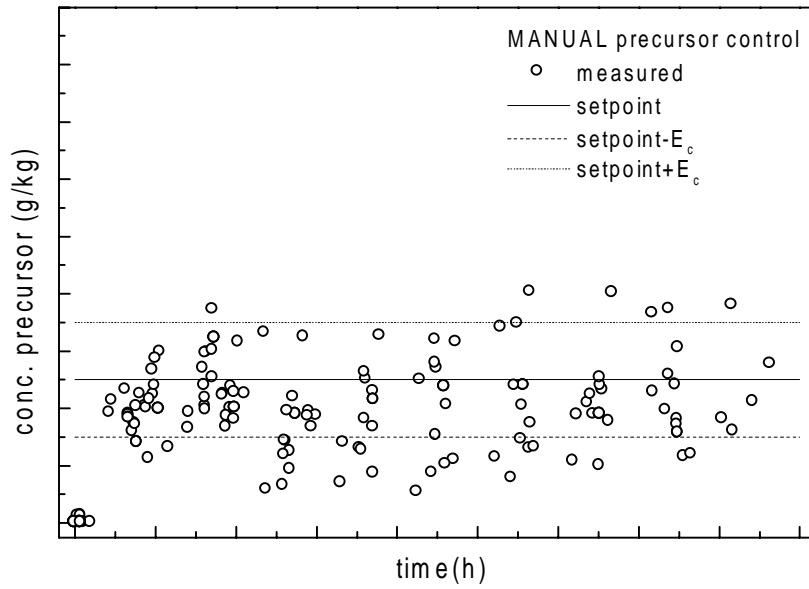Fig. 9. Concentration of precursor for 17 fermentations controlled by BUBE

Fig. 10. Concentration of precursor for 12 fermentations controlled manually

The practical advantage of such a controller is that the process can be kept much closer to the desired optimal process trajectories. Hence, the data provide a better base for the decisions to be made on the experimental results. Secondly, the manpower in the laboratories can concentrate more on conceptual improvements of the process instead of acting as living process controllers.

The root mean square deviations of the ammonia concentrations from their set-points decreased by about 7% with the introduction of the new control system. The corresponding deviations of the precursor concentrations decreased by about 14%.

The Hardware/Software configuration proved to be stable enough to use it routinely. The control system revealed to be robust, as a consequence of its efficient feedforward/feedback algorithm and on-line learning.

However, it is worth noting that while such a system may allow to reduce the manpower in the laboratory, the number of on-line or off-line measurements cannot be further decreased since relevant measurement information about the actual process is indispensible.

NOMENCLATURE

a	scaling factor for the precursor estimation error
a_1, b_1	time-invariant parameters in the fixed carbon correlation for estimating the ammonia consumption rate
$a_2(t), b_2(t)$	time-varying parameters in the fixed carbon correlation for estimating the precursor consumption rate
b	scaling factor for the ammonia estimation error
C_{fs}	substract concentration in the substract feed into the fermenter
C_n	ammonia concentration in the broth
$C_{n,e}$	measured ammonia concentration
$C_{n,fn}$	ammonia concentration in the feed of ammonia into the fermenter
$C_{n,sp}$	setpoint ammonia concentration
C_{pa}	precursor concentration in the broth
$C_{pa,e}$	measured precursor concentration
$C_{pa,fpa}$	precursor concentration in the feed of precursor into the fermenter
$C_{pa,sp}$	setpoint precursor concentration
CPR	carbon dioxide production rate by the mould
E	least-squares error
E_c	maximum error allowed for concentrations estimations
E_n	maximum ammonia concentration deviation to the setpoint
E_{pa}	maximum precursor concentration deviation to the setpoint
E_r	maximum error allowed for consumption rates estimations
f_c	rate of carbon fixed by the mould
$F_{i,fb}$	feed rate of component i=ammonia,precursor given by the feedback controller
$F_{i,ff}$	feed rate of component i=ammonia,precursor given by the feedforward controller
F_n	ammonia feed rate into the fermenter
$F_{n,rec}$	recommended ammonia feed rate
F_{pa}	precursor feed rate into the fermenter
$F_{pa,rec}$	recommended precursor feed rate
F_s	substract feed rate into the fermenter
F_{tot}	total feed into the fermenter
M_s	substract C-molar weigh
OUR	oxygen uptake rate by the mould
P	number of experimental measurements of ammonia and precursor concentrations
R_n	ammonia consumption rate
$R_{n,fc}$	ammonia consumption rate estimation by using the fixed carbon correlation
R_{pa}	precursor consumption rate
$R_{pa,fc}$	precursor consumption rate estimation by using the fixed carbon correlation

RQ	respiration quotient
R_s	substract consumption rate
t	independent variable time
W	vector of all the parameters involved in the hybrid model
W_B	broth weight
Δt	sampling time for off-line measurements
α, β	scaling factors derived from the variances of precursor and ammonia concentrations respectively, used to scale the estimation error E
$\tau(h)$	time constant

REFERENCES

Aström, K.J. and B. Wittenmark (1989).Adaptive Control. Addison-Wesley, Reading, MA

Bajpai, R.K., M. Reuss (1981). Evaluation of feeding strategies in carbon-regulated secondary metabolite production through mathematical modelling. *Biotechnol. Bioeng.,* **23**, pp 717-738

Constantinides, A., J.L. Spencer, E.L. Gaden (1970). Optimization of batch fermentation processes: II. Optimum temperature profiles for batch penicillin fermentation. *Biotech. Bioeng.*, **12**, pp. 1081-1098.

Court, J.R., S.J. Pirt (1981). Carbon and nitrogen-limited gowth of penicillium chrysogenum in fed batch culture: the optimal ammonium ion concentration for penicillin production. *J.Chem.Technol.Biotechnol.*, **31**, pp. 235-240

DiMassimo, C., G.A. Montague, M.J. Willis, M.T. Tham, A.J. Morris (1992). Towards improved penicillin fermentation via artificial neural networks. *Comp. Chem. Eng*., **16**, pp. 283-291

Feyo de Azevedo, S., B. Dahm, F.R. Oliveira (1997). Hybrid Modelling of Biochemical Processes: A comparison with the conventional approach. *Comp. Chem. Eng.*, **21,** Suppl., pp. 751-756

Garcia, C.E., D. M. Prett, M. Morari (1989). Model predictive control: Theory and practice – A survey. *Automatica*, **25**, pp. 335-348

Gately, E. (1996), Neural networks for financial forecasting, Wiley, New York

Hunt, K. J., D. Sbarbaro , R. Zwikowski, P.J. Gawthrop (1992). Neural networks for control systems: A survey. *Analytica*, **28**, pp. 1083-1112

Kresta, J.V., J.V. Macgregor (1991). Multivariate statistical monitoring of process operating performance. *Can. J. Eng.*, **69**, pp. 35-47

Leonard, J., M.A. Kramer (1990). Improvement of the backpropagation algorithm for training neural networks. *Comp. Chem. Eng.*, **14**, pp. 337-341

Lurie L.M., T.P. Verkhovsteva, A.I. Orlova, M.M. Levitor (1976). Technology of drug manufacture. Nitrogen nutrition as a factor in the intensification of penicillin synthesis. *Pharm. Chem. J.*, **10**, pp. 218-222

Modak, J.M., H.C. Lim (1987). Feedback optimization of fed-batch fermentations. *Biotechn. Bioeng*., **30**, pp. 528-540

Nielsen, J., J. Villadsen (1994). Bioreaction Engineering Principles. Plenum Press, New York

Oliveira, R., R. Simutis, S. Feyo de Azevedo, A. Lübbert (1998). Hybnet, an Advanced Tool for Process Optimization and Control. . 7th Int. Conference on Computer Applications in Biotechnology – CAB7, Osaka, Japan, May 31-June 4, 1998 (in press)

Pollard, J.F., M.R. Broussard, D.B. Garrison, K.Y. San (1992). Process identification using neural networks. *Comp. Chem. Eng.*, **16**, pp. 253-270

Preusting, H., J. Noordover, R. Simutis, A. Lübbert (1996). The use of Hybrid modeling for the optimization of the penicillin fermentation process. *Chimia*, **50(9)**, pp. 416-417.

Psichogios, D.C., L.H. Ungar (1992). A hybrid neural network - first principles approach to process modeling. *AIChE J.*, **38**, pp. 1499-1511.

Schubert, J., R. Simutis, M. Dors, I. Havlik, A. Lübbert (1994). Bioprocess optimization and control: Application of hybrid modelling. *J.Biotechn.*, **35**, pp. 51-68

Simutis, R., M. Dors, I. Havlik, A. Lübbert (1995). Artificial neural networks of improved reliability for industrial process supervision. Proc. 6th Int. Conference on Computer Applications in Biotechnology - CAB6, Garmisch-Partenkirchen, Germany

Simutis, R., R. Oliveira, M. Manikowski, S. Feyo de Azevedo , A. Lübbert (1997). How to increase the performance of models for process optimization and control. *J. Biotechnol.,* **59**, pp. 73-89

Thomson, M.L., M.A. Kramer (1994). Modeling chemical processes using prior knowledge and neural networks. *AIChE J.*, **40**, pp. 1328 - 1340.

Thorn, J.A., M.J. Johnson, (1950). Precursors for aliphatic penicillins. *J.Amer.Chem.Soc.*, **72**, pp. 2052-2058

Werbos, P.J. (1990), Backpropagation through time: what it does how to do it?, *Proc.IEEE*, **78**, pp. 1550-1560

Chapter 5

A Study on the Convergence of Observer-based Kinitics Estimators in Stirred Tank Reactors

Abstract. In this paper a model-based parameter estimator is proposed for the on-line estimation of reaction rates in stirred tank bioreactores. A particular attention is given to the stability requisites and the dynamics of convergence of the estimates to the true values. These two fundamental issues are discussed in relation to the tuning procedure of the gain parameters. The application of the algorithm is illustrated with a simple microbial growth cultivation process.

1 INTRODUCTION

Model-based state observation and parameter estimation represent fundamental tools for control and monitorization of biotechnological processes. Dochain and Bastin (1990) established a general theoretical framework for the analysis of bioreactor dynamics. The concept of a general state space dynamical model for bioreactors was proposed

$$\frac{d\xi}{dt} = K\varphi(\xi) - D\xi + F - Q \tag{1}$$

where ξ is the state vector (the set of n component concentrations), K an n×m yield coefficients matrix, D the dilution rate, F the feed rate vector with dim(F)=n and Q the gaseous outflow rate vector with dim(Q)=n.

This general dynamical model constitutes the key element for the design of State Observers and Parameter Estimators. A wide class of problems is covered depending upon the degree of knowledge of the process model. Situations like 'unknown (some or all) yield coefficients', or 'unknown or partially known kinetics model' are extensively studied.

The need of a kinetics model constitutes a key point in this discussion. The reaction rates φ are most often a very complex relation of the operating conditions and of the state of the process. The construction of a suitable kinetics model may constitute a very difficult task, if not an impossible one. As such, there is a clear incentive to design monitoring and control algorithms for bioprocesses with a *minimal modelling of the kinetics.* This concept of minimal kinetic modelling is discussed by Bastin and Dochain (1990) and formalized as follows:

$$\varphi(\xi) = H(\xi)\rho(\xi) \tag{2}$$

being H(ξ) a m×r matrix of known functions of the state, and $\rho(\xi)$ a vector of r unknown functions of the state.

With this definition the general dynamical model is rewritten giving:

$$\frac{d\xi}{dt} = KH(\xi)\rho(\xi) - D\xi + F - Q \tag{3}$$

In this work, an algorithm based on model (3) is proposed which aims at the on-line estimation of the unknown functions of the state $\rho(\xi)$ by considering them as unknown time-varying parameters.

2 STATEMENT OF THE ESTIMATION PROBLEM

The algorithm presented below addresses a class of estimation problems which can be defined in the following four points:

i) The biothecnological process can be described by the general state space dynamical model (3)

ii) The yield coefficients (matrix K) are known and constant

iii) The dilution rate D, the feed rates F and the gaseous outflow rates Q are measured on-line.

iv) The state variables are known on-line either by measurement or by means of a state observer.

As such, the scope of the algorithm will be the on-line estimation of $\rho(\xi)$ from the on-line knowledge of D, F, Q and ξ.

3 THE ALGORITHM

The proposed algorithm is constituted by a state observer which provides estimates of "r" state space variables (ξ_1) and an additional equation for the updating of the "r" parameter estimates. The observation error, which is supposed to reflect the mismatch between the estimated parameters and its true values, is used as the driven force in the updating law. They are stated as follows:

$$\frac{d\hat{\xi}_1}{dt} = K_1 H(\hat{\xi}_1, \xi_2)\hat{\rho} - D\hat{\xi}_1 + F_1 - Q_1 - \Omega_1(\xi_1 - \hat{\xi}_1) \tag{4a}$$

$$\frac{d\hat{\rho}}{dt} = \Omega_2(\xi_1 - \hat{\xi}_1) \tag{4b}$$

where $\hat{\xi}_1$ denotes the on-line estimate of ξ_1 and $\hat{\rho}$ the on-line estimate of $\rho(\xi)$. Ω_1 and Ω_2 are square (r×r) tuning matrices for the control of stability and tracking properties of the algorithm.

4 STABILITY ANALYSIS

The dynamics of the observation error and of the tracking error are obtained by subtracting eqn. (3) from eqn (4a) leading to the following non-linear system:

$$\frac{d(\xi_1 - \hat{\xi}_1)}{dt} = K_1 \left[H(\xi_1, \xi_2)\rho - H(\hat{\xi}_1, \xi_2)\hat{\rho} \right] - D(\xi_1 - \hat{\xi}_1) - \Omega_1(\xi_1 - \hat{\xi}_1) \tag{5a}$$

$$\frac{d(\rho - \hat{\rho})}{dt} = -\Omega_2(\xi_1 - \hat{\xi}_1) + \frac{d\rho}{dt} \tag{5b}$$

where $\dot{\rho}$ is considered as an external perturbance.

The point $\hat{\xi}_1 = \xi_1$ and $\hat{\rho} = \rho$ is an equilibrium point of the unperturbed system. A linear approximation of the unperturbed system around $\hat{\xi}_1 = \xi_1$ and $\hat{\rho} = \rho$ gives:

$$\frac{dE}{dt} = AE \tag{6}$$

with

$$E = \begin{bmatrix} \xi_1 - \hat{\xi}_1 \\ \rho - \hat{\rho} \end{bmatrix} \qquad A = \begin{bmatrix} C(\hat{\xi}_1, \xi_2, \hat{\rho}) - \Omega_1 & K_1 H(\hat{\xi}_1, \xi_2) \\ -\Omega_2 & 0 \end{bmatrix}$$

being $C(\hat{\xi}, \hat{\rho})$ defined by

$$C(\hat{\xi}_1, \xi_2, \hat{\rho}) = K_1 \left[\frac{\partial \left[H(\xi_1, \xi_2) \hat{\rho} \right]}{\partial \xi_1} \right]_{\xi_1 = \hat{\xi}_1} - DI_N \tag{7}$$

From the direct method of Lyapunov, it follows that the unperturbed system is exponentially stable if

C1. the eingenvalues of matrix A have strictly negative real parts.

In addition the perturbed system is globally stable (i.e. the output error is bounded for all t) if $\dot{\rho}$ is a continuously differentiable bounded function. The conditions under which this is verified were established by Dochain and Bastin (1990)

C2. The dilution rate is bounded below:

$$0 < D_{min} \leq D(t) \qquad \forall_t \tag{8}$$

C3. The feed rates are bounded :

$$0 < F_i(t) \leq F(t) \qquad \forall_i \forall_t \tag{9}$$

C4. Each reaction involves at least one reactant that is neither a catalyst nor an autocatalyst.

C5. $\rho(\xi)$ is a differentiable function of ξ.

5 TUNING OF THE GAIN MATRICES

The gain matrices are computed on-line in order to impose a second-order dynamics of convergence of $\hat{\rho}$ to $\rho(\xi)$:

$$\tau_i^2 \frac{d^2 \hat{\rho}_i}{dt^2} + 2\zeta_i \tau_i \frac{d\hat{\rho}_i}{dt} + \hat{\rho}_i = \rho_i \quad i=1,...,m \tag{10}$$

under the constraints of the linearised tangent error model., which gives:

$$\Omega_1(\hat{\xi}_1, \xi_2, \hat{\rho}) = C(\hat{\xi}_1, \xi_2, \hat{\rho}) + diag\left\{\frac{2\zeta_i}{\tau_i}\right\} \tag{11a}$$

$$\Omega_2(\hat{\xi}_1, \xi_2) = \left[K_1 H(\hat{\xi}_1, \xi_2)\right]^{-1} diag\left\{\frac{1}{\tau_i^2}\right\} \tag{11b}$$

with this tuning, condition C1 is automatically verified provided that τ_i and ζ_i are strictly positive real constants.

6 TESTING THE ALGORITHM

In this section, the use of algorithm (4) is illustrated through a simple application: the estimation of a microbial specific reaction rate in a simple biological culture which involves a single biomass (X) growing on a single substrate (S) and yielding a single product (P). To test the capability of the algorithm at imposing a second order dynamics of convergence, the specific reaction rate is assumed to be a square wave signal.

The reaction scheme is stated as follows:

$$S \xrightarrow{\varphi} X + P \tag{12}$$
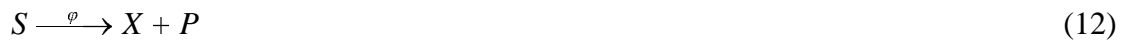
The process dynamics in a fed-batch fermenter are described by eqn. (13).

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ P \end{bmatrix} = \begin{bmatrix} 1 \\ -k_1 \\ k_2 \end{bmatrix}\varphi - D\begin{bmatrix} X \\ S \\ P \end{bmatrix} + \begin{bmatrix} 0 \\ DS_{in} \\ 0 \end{bmatrix} \tag{13}$$

where D is the dilution rate ($D=F/V$ being F the input flow rate and V the solution volume in the fermenter), S_{in} the substrate concentration in the feed.

The reaction rate is defined as follows:

$$\varphi = XS\alpha \tag{14}$$

being α the specific reaction rate.

In the present case the objective is the on-line estimation of the time-varying specific reaction rate from the on-line knowledge of X, S, P, S_{in}, V and F.

The application of algorithm (4) with $\xi_1 = X$ leads to the following two eqns.

$$\frac{d\hat{X}}{dt} = \hat{X}S\hat{\alpha} - D\hat{X} + \omega_1(X - \hat{X}) \tag{15a}$$

$$\frac{d\hat{\alpha}}{dt} = \omega_2(X - \hat{X}) \tag{15b}$$

being ω_1 and ω_2 given by

$$\omega_1 = S\hat{\alpha} - D + \frac{2\zeta}{\tau} \tag{16a}$$

$$\omega_2 = \frac{1}{\hat{X}S\tau^2} \tag{16b}$$

The results obtained are shown in Figs (1-10). The dotted line represent the true specific reaction rate while the full line represent the respective estimate. The accuracy of the estimates can be accessed from the ITAE error index (ITAE - integral of time-weighted absolute errors) given in the legend. The influence of ζ can be accessed from the plots in Figs. (1-5) where τ is kept at a constant value of 0.15 while ζ assumes 0.25, 0.75, 1., 1.25, 1.5.
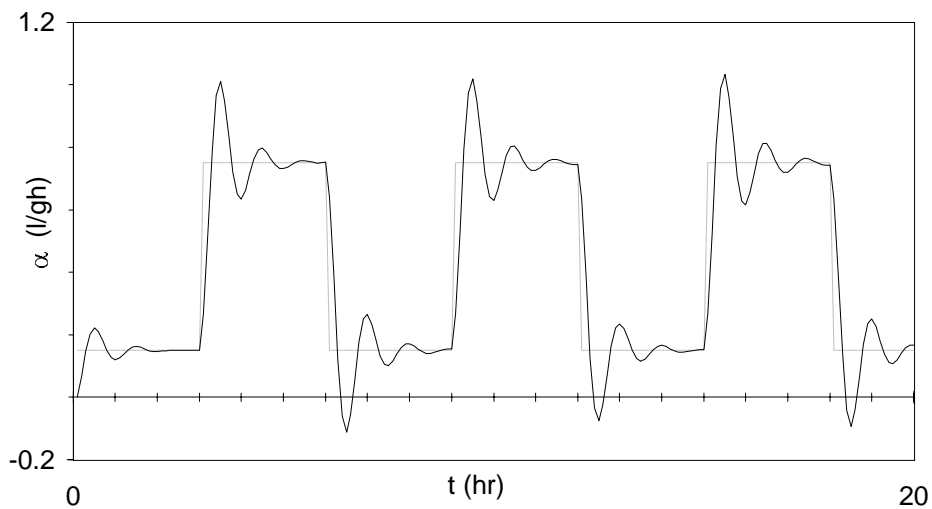
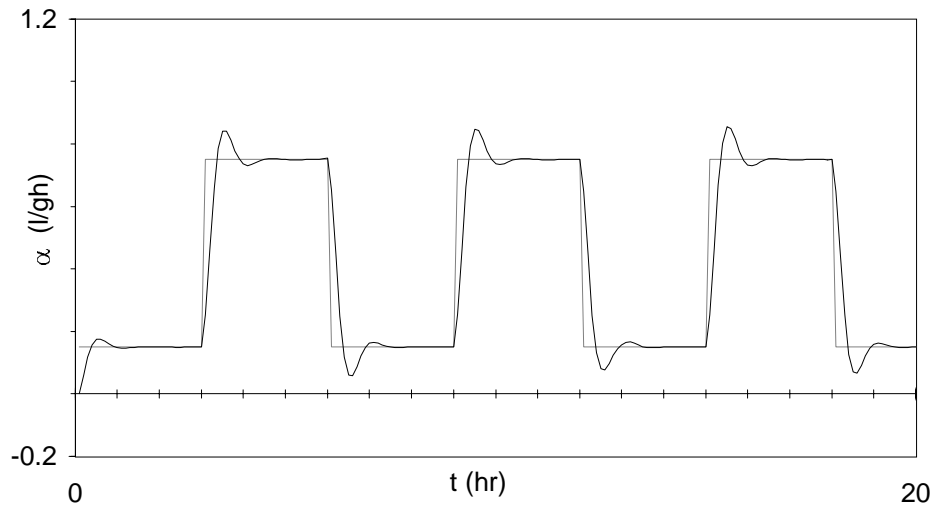Fig. 1 - Results obtained with τ=0.15 and ζ=0.25 (ITAE=14.0)
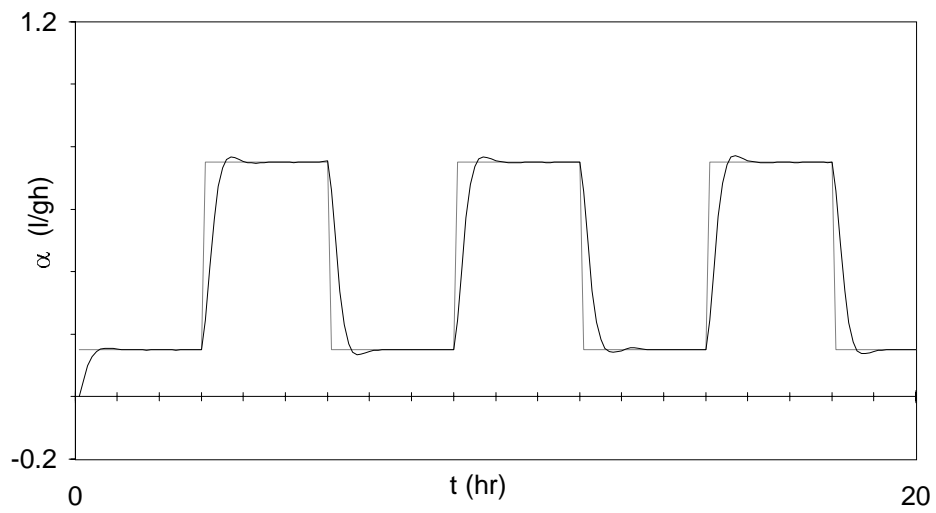
Fig. 2 - Results obtained with τ=0.15 and ζ=0.5 (ITAE=8.1)

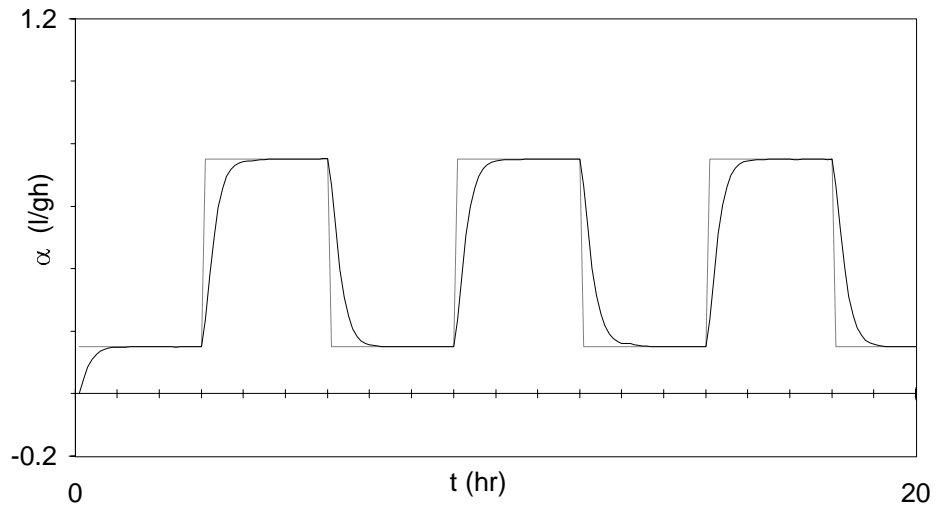Fig. 3 - Results obtained with τ=0.15 and ζ=0.75 (ITAE=7.7)

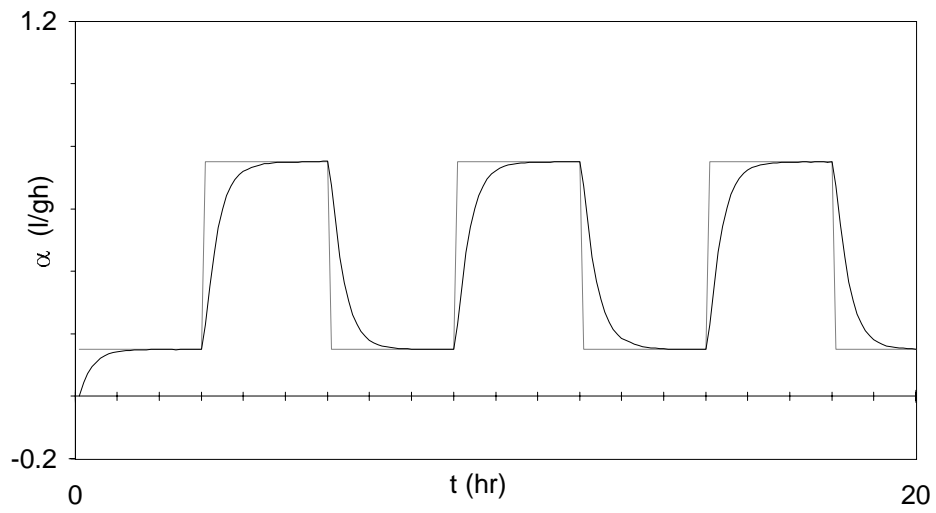Fig. 4 - Results obtained with $\tau=0.15$ and $\zeta=1.0$ (ITAE=9.9)

Fig. 5 - Results obtained with $\tau=0.15$ and $\zeta=1.25$ (ITAE=13.0)
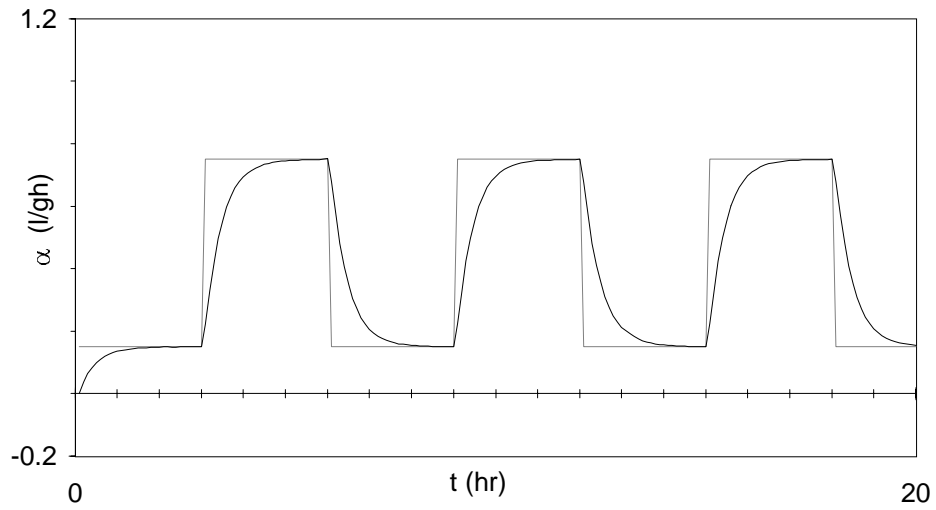
Fig. 6 - Results obtained with τ=0.15 and ζ=1.5 (ITAE=16.0)

The influence of τ can be accessed from the plots in Figs. (7-10) where ζ is kept at a constant value of 0.8 while τ assumes 0.15, 0.1, 0.05 and 0.01.
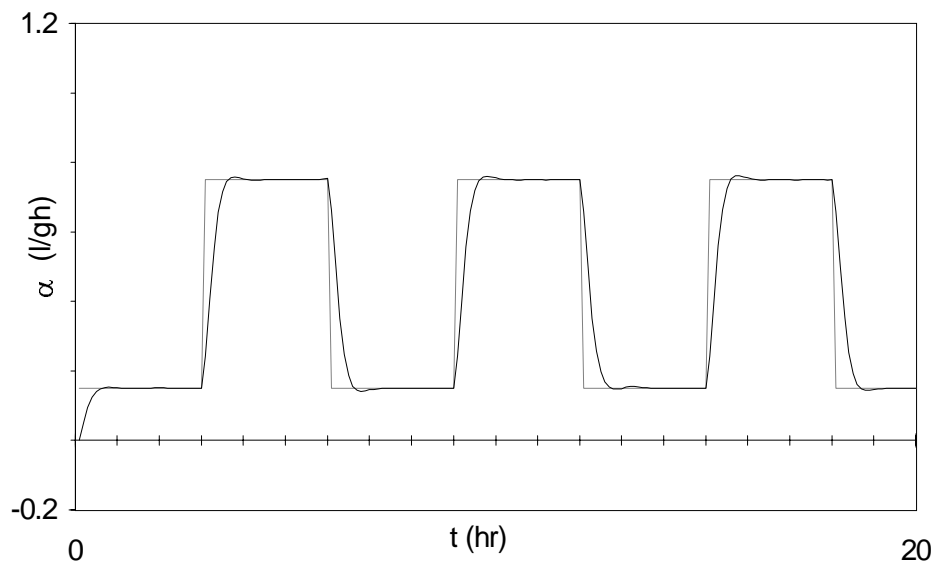

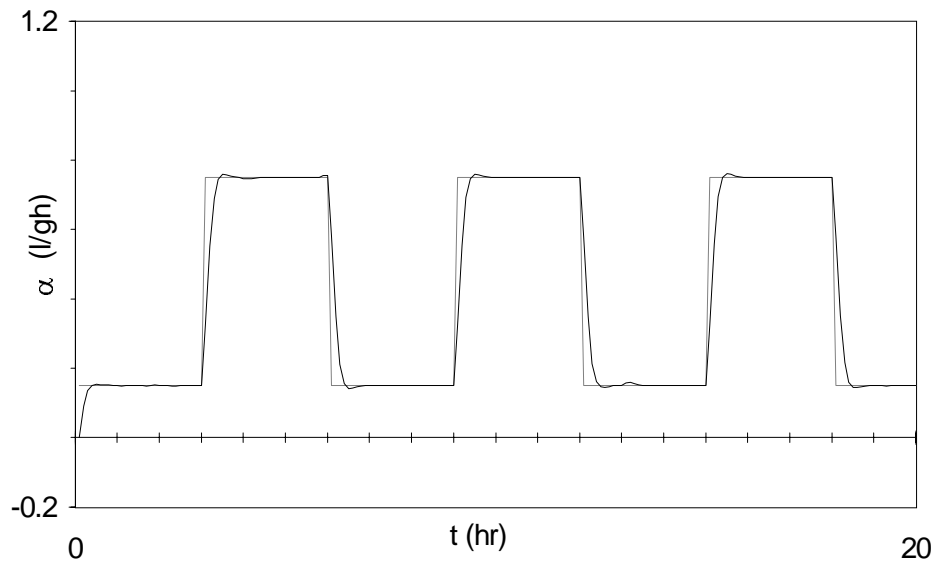Fig. 7 - Results obtained with τ=0.15 and ζ=0.8 (ITAE=7.9)

Fig. 8 - Results obtained with τ=0.1 and ζ=0.8 (ITAE=4.6)
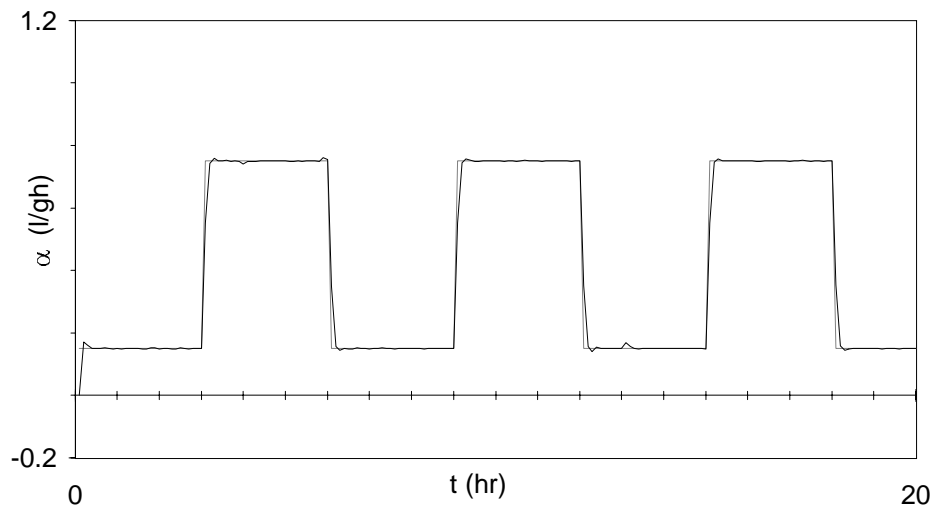

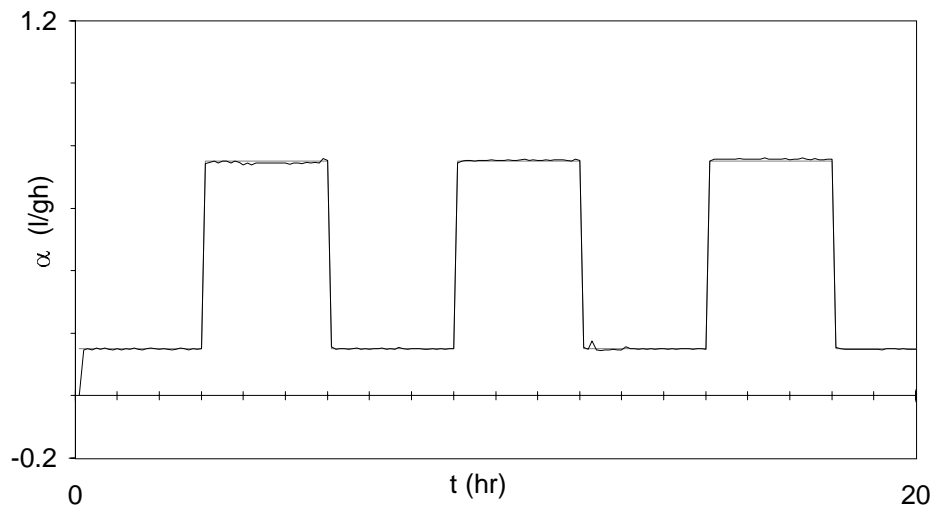Fig. 9 - Results obtained with τ=0.05 and ζ=0.8 (ITAE=1.5)

Fig. 10 - Results obtained with τ=0.01 and ζ=0.8 (ITAE=0.73)

These results suggest that the dynamics of convergence of $\hat{\alpha}$ to α have characteristics which are in agreement of a typical second order dynamic response to a step input. It is shown that decreasing τ the response becomes faster while decreasing ζ the response turns to be more oscillatory. Another evidence of this agreement is that, as given by the plots in Figs (1-6), ζ=1 defines the frontier between oscillatory and non-oscillatory responses.

NOMENCLATURE

C	oxygen concentration
CTR	carbon dioxide transfer rate
D	dilution rate
E	ethanol concentration
F	mass feed rate vector
G	carbon dioxide concentration
$H(\xi)$	(m×r) matrix of known functions of the state
K	yield coefficients matrix
k_i	yield coefficients
OTR	Oxygen transfer rate
Q	rate of mass removel in gaseous form vector
S	glucose concentration
S_{in}	glucose concentration in the feed
T	sampling period
X	biomass concentration

Greek letters

φ	reaction rates vector
μ	specific growth rates vector
$\hat{\mu}$	vector of estimated specific growth rates
μ_s^o	specific growth rate for the respiratory growth on glucose pathway
μ_s^r	specific growth rate for the fermentative growth on glucose pathway
μ_e^o	specific growth rate for the respiratory growth on ethanol pathway
$\rho(t)$	vector of completely unknown time-varying parameters
$\hat{\rho}$	vector of estimatives of $\rho(t)$
τ_i	natural period of oscillation
ω_i, γ_i	diagonal elements of Ω and Γ
ξ	state space vector
$\hat{\xi}$	estimated state vector
ξ_1	measured state space vector
ξ_2	non-measured state space vector

$\hat{\xi}_2$	estimated vector of nonmeasured state variables	88
ζ_i	damping coefficient	
Ω, Γ	gain matrices	

REFERENCES

Bastin, G., Dochain, D. (1990) 'On-Line Estimation and Adaptive Control of Bioreactors', Elsevier, Amsterdam.

Pomerleau, Y. and Perrier, M. (1990) 'Estimation of Multiple Specific Growth Rates in Bioprocesses', AIChE Journal, Vol. 36, n. 2, 207-215.

Pomerleau Y. (1990), "Modélisation et contrôle d'un procédé fed-batch de culture des levures à pain", Ph. D. Thesis, Ecole Polytechnique de Montréal, Canada.

Sonnleitner, B. and Käppeli, O. (1986) 'Growth of Saccharomyces cerevisiae is controlled by its Limited Respiratory Capacity: Formulation and Verification of a Hypothesis', Biotech. Bioeng., Vol. 28, June, pp.927-937.

D.G. Luenberger (1971). An introdution to observers. IEEE Trans. Automatic Control, vol. AC-16, pp. 596-603.

S. Feyo de Azevedo, P.Pimenta, F. Oliveira, E. Ferreira, 1992, "Studies on On-Line State and Parameter Estimation through a Real-Time Process Simulator', in Karim, M. N. and Stephanopoulos, J. (Eds.), Modeling and Control of Biotschnical Processes, IFAC Symp. Series 10, PP 453-458, Pergamon Press N.Y.

Chapter 6

On-line State Observation and Reaction Rates Estimation in a Baker's Yeast Cultivation Process

Abstract. In this paper algorithms for state observation and kinetics estimation are developed and applied to a baker's yeast fed-batch cultivation process. An important design condition was to keep the number of required on-line measurements as low as possible. The overall estimation scheme aims at the estimation of 3 state variables and 3 specific growth rates requiring on-line measurements of dissolved oxygen, dissolved carbon dioxide and off-gas analysis. A great deal of attention is given to the estimation problem of reaction kinetics. In this respect a new algorithm is proposed -the Second Order Dynamics Estimator (SODE)- and compared to an Observer Based Estimator (OBE). Stability and dynamics of convergence are issues subject of detailed analysis. The relations between the numerical implementation and stability are also studied. It is shown that a discrete-time formulation poses additional stability constrains. These can be easily overcome by the use of a robust variable step integration algorithm. It was concluded that the OBE has two main disadvantages: i) the tuning of the design parameters must be done on a trial-and-error basis, while in the SODE the user can set a 2nd order dynamics of convergence from estimated kinetics to "true" kinetics, and ii) the dynamics of convergence of the OBE are time-varying while in the case of the SODE this response is time-invariant.

1 INTRODUCTION

Two of the major problems which hinder the implementation of advanced monitoring and control methods in bioreactors are the difficulty of modelling the growth kinetics of microorganisms and the absence of cheap and reliable sensors capable of providing direct real time measurements of the state variables.

The design and implementation of Software Sensors provides a suitable answer to cope with the lack of instrumental sensors. Software Sensors are algorithms for the on-line estimation of the state variables and the parameters which are not measurable in real time, from related measurements which are more easily accessible.

The mathematical description of microorganisms growth kinetics is a critical issue in bioprocess modelling. Quite often kinetic models are based on unstructured and non-segregated cell models. Unfortunately, in many cases, such models are not accurate enough to solve the problems in study. The other critical issue is related to the identification of kinetic parameters. Parameter identification requires a careful and expensive experimental planing. As such, there is a clear incentive to develop algorithms for state estimation and parameters estimation while avoiding the knowledge of the underlying kinetic model.

In the present paper, reaction kinetics estimation from related on-line measurements is a central issue. In this respect a new algorithm -the Second Order Dynamics Estimator (SODE)- is proposed, which aims at imposing 2th order dynamics of convergence of estimated kinetics to the corresponding "true" kinetics. The properties of the SODE are studied and compared to the Observer-Based Estimator (OBE) proposed by Dochain and Bastin (1990). Stability and dynamics of convergence are subjects of detailed analysis. The relations between the numerical implementation and stability is also studied.

The behaviour of these algorithms is carefully analysed by the application to a baker's yeast fed-batch cultivation process. A general estimation scheme is proposed, where 3 state variables (biomass, glucose and ethanol concentrations in the broth) and 3 kinetics (specific growth rates related to glucose oxidation, glucose fermentation, and ethanol oxidation) are estimated, using only on-line measurements of 2 state variables (concentrations of dissolved oxygen and of dissolved carbon dioxide) and off-gas analysis.

2 GENERAL FRAMEWORK

Bastin and Dochain (1990) proposed a methodology for state and parameter estimation based upon a general dynamical model for stirred-tank bioreactors:

$$\frac{d\xi}{dt} = K\varphi(\xi) - D\xi + F - Q \tag{1}$$

where ξ is the state vector (the set of n component concentrations), K an n×m yield coefficients matrix, D the dilution rate, F the feed rate vector with dim(F)=n and Q the gaseous outflow rate vector with dim(Q)=n.

One question of major concern is the design of Software Sensors avoiding kinetic modelling. In eqn. (1) the reaction rates $\varphi(\xi)$ were defined as:

$$\varphi_i(\xi) = h_i(\xi)\rho_i(\xi) \qquad\qquad i=1,...,m \qquad\qquad (2)$$

where $h_i(\xi)$ is a known function of the state while $\rho_i(\xi)$ is an unknown function of the state. Or more generally:

$$\varphi(\xi) = H(\xi)\rho(\xi) \qquad\qquad\qquad (3)$$

with $H(\xi)$ an *m×r* matrix of known functions of the state and $\rho(\xi)$ a vector of r unknown functions of the state.

The strategy is to insert into $H(\xi)$ only the prior knowledge regarding the kinetics and then to consider $\rho(\xi)$ as a completely unknown "time-varying" parameter which can be estimated on-line through the use of parameter estimators.

In the following sections 3 algorithms are presented for state observation and kinetics estimation. These algorithms were developed assuming the general structure of the dynamical model (1). They are later used in section 3 for designing a complete state estimation and kinetics estimation scheme for a baker's yeast cultivation process.

2.1 The Observer-Based Estimator (OBE)

For the estimation of reaction rates from the on-line knowledge of the state variables, when the yield coefficients are known and constant, Bastin and Dochain (1990) proposed an observer-based estimator which is expressed by:

Observer-Based Estimator (OBE)

$$\frac{d\hat{\xi}}{dt} = KH(\xi)\hat{\rho} - D\xi + F - Q - \Omega(\xi - \hat{\xi}) \qquad\qquad (4a)$$

$$\frac{d\hat{\rho}}{dt} = \left[KH(\xi)\right]^{\mathrm{T}} \Gamma(\xi - \hat{\xi}) \qquad\qquad (4b)$$

---

where Ω and Γ are square n×n matrices which are design parameters at the disposal of the user for the control of stability and tracking properties of the algorithm.

The basic idea is to use a state observer (4a) to estimate an observation error $(\xi - \hat{\xi})$ which is supposed to reflect the mismatch between $\hat{\rho}(\xi)$ and $\rho(\xi)$ and then use it as the driven force in the updating law (4b).

2.1.1 Stability analysis. The continuous error system can be obtained defining the observation error $\tilde{\xi} = \xi - \hat{\xi}$ and the tracking error $\tilde{\rho} = \rho - \hat{\rho}$ and subtracting eqn. (1) by eqn. (4a):

$$\frac{dE}{dt} = AE + B \tag{5}$$

with

$$E = \begin{bmatrix} \tilde{\xi} & \tilde{\rho} \end{bmatrix}^T \qquad A = \begin{bmatrix} \Omega & KH(\xi) \\ -[KH(\xi)]^T \Gamma & 0 \end{bmatrix} \qquad B = \begin{bmatrix} 0 & \dfrac{d\rho}{dt} \end{bmatrix}^T$$

The dynamics of the error system are linear time-varying (LTV) due to the presence of the state variables in matrix A.

The global stability of the error system (5) is ensured if the disturbance vector B is bounded and if the unforced system is exponentially stable .

A BIBS (Bonded Input Bounded State) analysis of the dynamic model (1) (Dochain and Bastin, 1990) gives that if:

C1. The dilution rate is bounded below:

$$0 < D_{min} \le D(t) \qquad\qquad \forall_t \tag{6}$$

C2. The feed rates are bounded :

$$0 < F_i(t) \le F(t) \qquad\qquad \forall_i \forall_t \tag{7}$$

C3. Each reaction involves at least one reactant that is neither a catalyst nor an autocatalyst.

then the state variables ξ are positive and bounded for all t. If additionally

C4. $\rho(\xi)$ is a differentiable function of ξ.

then the boundness of the disturbance vector B is ensured. On the other hand, if:

C5. Ω is a n×n constant matrix with all its eigenvalues having strictly real parts.

C6. Γ is a n×n constant matrix such that the matrix $\Omega^{\mathrm{T}}\Gamma + \Gamma\Omega$ is negative definite.

C7. $KH(\xi)$ is a persistently exciting matrix.

Then the unforced error system is exponentially stable and therefore, the perturbed error system (5) is globally stable.

2.1.2 Tuning of design parameters. As stated above Ω and Γ are square n×n matrices which are design parameters at the disposal of the user for the control of stability and tracking properties of the algorithm, thus playing a decisive roll on the performance of the estimator. A common choice is to take:

$$\Omega = diag\{-\omega_i\} \quad \Gamma = diag\{\gamma_i\} \qquad i=1,...,n \tag{8}$$

where ω_i and γ_i are 2×n strictly positive real constants. With this choice conditions C4 and C5 are automatically verified and the tuning procedure reduces to the calibration by trial and error of 2×n scalar constants.

2.1.3 Reduced-order Observer-Based Estimator. The observer-based estimator (4) is based on the full dynamical model of the process. In practice this is not always necessary. It is often sufficient to design the estimator from a subset of the state equations provided they involve all the r parameters which need to be estimated. In particular, under the following assumptions:

A1. There are r=m parameters which need to be estimated
A2. There is a subset of m equations of the full state space model that involve all the m parameters which need to be estimated:

$$\frac{d\xi_a}{dt} = K_a H(\xi)\rho(\xi) - D\xi_a + F_a - Q_a \tag{9}$$

A3. In eqn. 9 K_a is a m×m full-rank matrix

and by considering the transformation:

$$\psi = K_a^{-1}\xi_a \tag{10}$$

then eqn. (9) can be rewritten as:

$$\frac{d\psi}{dt} = H(\xi)\rho(\xi) - D\psi + K_a^{-1}(F_a - Q_a) \tag{11}$$

Based on this reduced-order reformulated process model, the observer-based estimator can be written as:

Reduced-order Observer-Based Estimator

$$\frac{d\hat{\psi}}{dt} = H\hat{\rho} - D\psi + K_a^{-1}(F_a - Q_a) - \Omega(\psi - \hat{\psi}) \tag{12a}$$

$$\frac{d\hat{\rho}}{dt} = H^T\Gamma(\psi - \hat{\psi}) \tag{12b}$$

2.2 Second Order Dynamics Estimator (SODE)

The Second Order Dynamics Estimator is a variant of the reduced order Observer-Based Estimator. They differ solely on the way the regressor in the updating law of $\hat{\rho}$ is stated (eqn. (12b)). It can be applied under assumptions A1 through A3 and additionally:

A4. The reaction rates can be defined by eqn. (2):

$$\varphi_i(\xi) = h_i(\xi)\rho_i(\xi) \qquad i=1,...,m \tag{2}$$

meaning that H(ξ) is a m×m diagonal matrix.

The second order dynamics based estimator is written as follows:

Second Order Dynamics Estimator (SODE)

$$\frac{d\hat{\psi}}{dt} = H\hat{\rho} - D\psi + K_a^{-1}(F_a - Q_a) + \Omega(\psi - \hat{\psi}) \tag{13a}$$

$$\frac{d\hat{\rho}}{dt} = \Gamma H^{-1}(\psi - \hat{\psi}) \tag{13b}$$

---

where Ω and Γ are square m×m matrices which as in the case of the Observer-Based Estimator, are design parameters for the control of stability and dynamics of convergence. Defining Ω=diag(ω_i) and Γ=diag(γ_i), eqns. (13) can be decoupled, giving:

$$\frac{d\hat{\psi}_i}{dt} = h_i\hat{\rho}_i - D\psi_i + U_i + \omega_i(\psi_i - \hat{\psi}_i) \tag{14a}$$

$$\frac{d\hat{\rho}_i}{dt} = \frac{\gamma_i}{h_i}(\psi_i - \hat{\psi}_i) \tag{14b}$$

with i=1,...,m

2.2.1 Stability analysis. The error system of eqns. (14) is a second order linear time variant (LTV) system:

$$\frac{d}{dt}\begin{bmatrix} \tilde{\psi}_i \\ \tilde{\rho}_i \end{bmatrix} = \begin{bmatrix} -\omega_i & h_i \\ -\gamma_i h_i^{-1} & 0 \end{bmatrix}\begin{bmatrix} \tilde{\psi}_i \\ \tilde{\rho}_i \end{bmatrix} + \begin{bmatrix} 0 \\ \dfrac{d\rho_i}{dt} \end{bmatrix} \tag{15}$$

with

$$\tilde{\psi}_i = \psi_i - \hat{\psi}_i \qquad\qquad \tilde{\rho}_i = \rho_i - \hat{\rho}_i$$

where $d\rho_i/dt$ is considered as an external persistent disturbance.

It is a standard result of the BIBO stability theory that a LTV system perturbed by an external disturbance is globally stable if the unperturbed system is uniformly asymptotically stable and the disturbance vector is bounded (Narendra and Annaswamy, 1989).

Conditions C1 through C4 assure the boundness of $d\rho_i/dt$ (Bastin and Dochain, 1990). Still, it remains to be proofed that the unforced system is uniformly asymptotically stable. The reader is referred to the appendix where this proof is presented.

2.2.2. Dynamics of convergence and tuning. Differentiating eqn. (14b) gives

$$\frac{d^2\hat{\rho}}{dt^2} = \frac{\gamma}{h}\frac{d\tilde{\psi}}{dt} - \gamma\frac{1}{h^2}\frac{dh}{dt}\tilde{\psi} \tag{16}$$

Combining eqns (16), (14b) and the first equation in (15) it follows that

$$\gamma^{-1}\frac{d^2\hat{\rho}}{dt^2} + \gamma^{-1}a(t)\frac{d\hat{\rho}}{dt} + \hat{\rho} = \rho \tag{17}$$

with a(t) given by eqn. (18).

$$a(t) = \omega(t) + h^{-1}\frac{dh}{dt} \tag{18a}$$

Supposing that the term

$$\phi(\xi) = \frac{1}{h(\xi)}\frac{dh}{dt} \tag{18b}$$

is known on-line corrupted by an error ε(t) (in practice the on-line knowledge of φ(ξ) requires an approximation to the time derivative dh/dt, and therefore, the estimated value $\hat{\phi}(\xi)$ is always corrupted by the approximation error ε(t)):

$$\hat{\phi}(\xi) - \phi(\xi) = \varepsilon(t) \tag{19}$$

an defining γ and ω(t) by:

$$\gamma = \frac{1}{\tau^2} \tag{20a}$$

$$\omega(t) = 2\zeta\sqrt{\gamma} - \hat{\phi}(\xi) = 2\zeta\sqrt{\gamma} - \phi(\xi) - \varepsilon(t) \tag{20b}$$

then eqn. (17) becomes:

$$\tau^2 \frac{d^2\hat{\rho}}{dt^2} + 2\zeta(t)\tau\frac{d\hat{\rho}}{dt} + \hat{\rho} = \rho \tag{21}$$

being ζ(t) related to the desired value ζ_d and the error ε(t) in the following way:

$$\zeta(t) = \zeta_d\left(1 - \frac{\tau\varepsilon(t)}{2\zeta_d}\right) \tag{22}$$

Hence, the conclusion is taken that each $\hat{\rho}(t)$ converges to it's true value ρ(t) with a second order dynamic response with constant natural period of oscillation τ and time-varying damping coefficient ζ(t).

Notice that defining ω(t) by eqn. (20b) implies that condition C7 which states that ω(t) must be always larger then -φ(ξ), is verified if $\frac{2\zeta_d}{\tau}$ is always larger then the approximation error ε(t). Notice also that as given by eqn. (22) this is equivalent to state that ζ(t) must be positive for all t.

2.2.3 Numerical implementation and stability. The numerical implementation of eqns. (14) requires a discrete-time formulation. The switch from the continuous-time equations to the discrete time versions leads to specific stability problems in which the integration step T plays an important role. One of the most popular approaches is the Euler discretisation of the continuous time equations. In this section, the implications of using an Euler descritisation in the stability of the SODE are analysed.

A forward Euler discretisation of eqns. (14) with ω(t) and γ given by eqns. (20) results in the following discrete-time equations:

$$\hat{\psi}_{t+1} = (1 - TD_t)\psi_t + Th_t\hat{\rho}_t + TU_t - T\omega_t(\psi_t - \hat{\psi}_t) \tag{23a}$$

$$\hat{\rho}_{t+1} = \hat{\rho}_t + \frac{T}{\tau^2 h_t}(\psi_t - \hat{\psi}_t) \tag{23b}$$

with

$$\omega_t = \frac{2\zeta}{\tau} + \frac{h_{t+1} - h_t}{Th_t} \tag{24}$$

The discrete error system is as follows

$$\tilde{\psi}_{t+1} = (1 + T\omega_t)\tilde{\psi}_t + Th_t\tilde{\rho}_t \tag{25a}$$

$$\tilde{\rho}_{t+1} = -\frac{T}{\tau^2 h_t}\tilde{\psi}_t + \tilde{\rho}_t + (\rho_{t+1} - \rho_t) \tag{25b}$$

which is equivalent to

$$E_{t+1} = AE_t + B_t \tag{26}$$

with

$$E_t = \begin{bmatrix} \tilde{\psi}_t \\ h_t \\ \tilde{\rho}_t \end{bmatrix} \qquad A = \begin{bmatrix} 1 + \dfrac{2\zeta}{\tau}T & T \\ -\dfrac{T}{\tau^2} & 1 \end{bmatrix} \qquad \begin{bmatrix} 0 \\ \rho_{t+1} - \rho_t \end{bmatrix}$$

The discrete-time error system (26) is linear time-invariant (LTI). The unforced system is exponentially stable (and hence the output error of (26) is bounded) if the eingenvalues of matrix A stay inside the unit circle. The eingenvalues of matrix A are given by

$$\lambda_1 = 1 - \frac{T}{\tau}\left(\zeta + \sqrt{\zeta^2 - 1}\right) \tag{27a}$$

$$\lambda_2 = 1 - \frac{T}{\tau}\left(\zeta - \sqrt{\zeta^2 - 1}\right) \tag{27b}$$

Case 1 - $\zeta<1$. The eingenvalues are two complex conjugate numbers

$$\lambda_1 = 1 - \frac{T}{\tau}\left(\zeta + i\sqrt{1 - \zeta^2}\right) \tag{28a}$$

$$\lambda_2 = 1 - \frac{T}{\tau}\left(\zeta - i\sqrt{1 - \zeta^2}\right) \tag{28b}$$

Hence, the stability condition applies

$$0 < \|\lambda_1\| = \|\lambda_2\| < 1 \tag{29}$$

which is equivalent to

$$0 < T < 2\zeta\tau \tag{30}$$

Case 2 - ζ=1. The eingenvalues are double given by

$$\lambda_1 = \lambda_2 = 1 - \frac{T}{\tau} \tag{31}$$

leading to the following stability condition

$$0 < T < \tau \tag{32}$$

Case 3 - ζ>1. The eingenvalues are two different real numbers given by eqns. (27). The stability condition is as follows:

$$0 < T < \frac{\tau}{\zeta \pm \sqrt{\zeta^2 - 1}} \tag{33}$$

As given by eqns. (30), (32) and (33) the range allowed for the integration step T is bounded and conditioned by the chosen τ and ζ. Notice also that these restrictions are the same if the analysis would have been carried out from the discrete version of eqn. (21).

2.3 The Luenberger-type asymptotic observer

Eqn. (1) can be divided in two partitions: the first one includes the equations related to the measured state variables (ξ_1); the second partition includes the equations related to the non-measured state variables (ξ_2). The dynamic model is rewritten as:

$$\frac{d\xi_1}{dt} = K_1\varphi(\xi) - D\xi_1 + F_1 - Q_1 \tag{34a}$$

$$\frac{d\xi_2}{dt} = K_2\varphi(\xi) - D\xi_2 + F_2 - Q_2 \tag{34b}$$

where K_1 (a full rank matrix), K_2, F_1, F_2, Q_1, Q_2 corresponds to the division of K, F and Q to each partition. Based on the transformation:

$$Z = \xi_2 - K_2K_1^{-1}\xi_1 \tag{35}$$

and on eqns. (34), the Luenberger-type asymptotic observer (Luenberger, 1971) is written as:

Luenberger-type Asymptotic Observer

$$\frac{dZ}{dt} = -DZ + (F_2 - Q_2) - K_2 K_1^{-1}(F_1 - Q_1) \tag{36a}$$

$$\xi_2 = Z + K_2 K_1^{-1} \xi_1 \tag{36b}$$

Notice that the number of measured state variables must be equal to the number of unknown reaction rates in vector $\varphi(\xi)$.

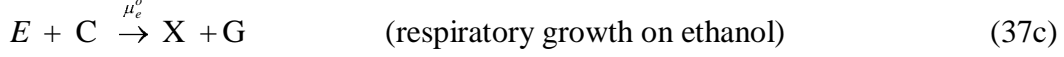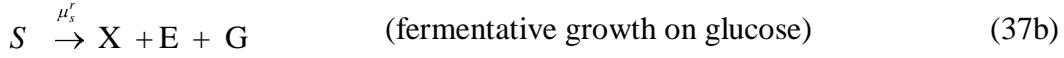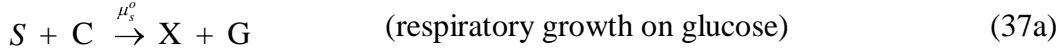## 3 STATE OBSERVATION AND KINETICS ESTIMATION IN A BAKER'S YEAST FED-BATCH CULTIVATION PROCESS

In section 2 general-use state observation and kinetics estimation algorithms were presented. They are going to be now applied to a baker's yeast fed-batch cultivation process. The objective is to develop a state observation and a specific growth rates estimation scheme requiring a minimum number of easily accessible on-line measurements. The discussion will flow though the following topics:

1) General dynamical model structure for a baker's yeast fed-batch cultivation process. This model will be the base on which the estimation algorithms are derived.
2) Statement of the estimation problem.
3) Derivation of the Luenberger-type asymptotic observer, the reduced order OBE and the SODE for the chosen on-line measurements

The overall estimation scheme will permit the estimation of 3 state variables (biomass, glucose and ethanol concentrations in the broth), 3 specific growth rates (related to glucose oxidation, glucose fermentation and ethanol oxidation) using measurements from 2 state variables (dissolved oxygen and dissolved carbon dioxide) and off-gas analysis (oxygen transfer rate and carbon dioxide transfer rate).

3.1 Dynamic model for a baker's yeast fed-batch cultivation process

Yeast growth is characterized by the following reaction scheme (Sonnleitner and Käppeli, 1986):

$$S + C \xrightarrow{\mu_s^o} X + G \qquad \text{(respiratory growth on glucose)} \qquad (37a)$$

$$S \xrightarrow{\mu_s^r} X + E + G \qquad \text{(fermentative growth on glucose)} \qquad (37b)$$

$$E + C \xrightarrow{\mu_e^o} X + G \qquad \text{(respiratory growth on ethanol)} \qquad (37c)$$

where S is glucose, C is oxygen, X is biomass, E is ethanol and G is carbon dioxide. μ_s^0 , μ_s^r and μ_e^o are three specific growth rates which reflects the capacity of the yeast to exploit three different catabolic pathways for energy and basic material sources.

The dynamical model for the fed-batch fermentor is obtained from a mass balance on the components, considering that the reactor is well mixed, the yield coefficients are constant and the dynamics of the gas phase can be neglected. The mass balances, in terms of concentration, are written as:

$$\frac{dX}{dt} = (\mu_s^o + \mu_r^s + \mu_e^o - D)X \qquad (38a)$$

$$\frac{dS}{dt} = D(S_{in} - S) + (-k_1\mu_s^o - k_2\mu_s^r)X \qquad (38b)$$

$$\frac{dE}{dt} = -DE + (k_3\mu_s^r - k_4\mu_e^o)X \qquad (38c)$$

$$\frac{dC}{dt} = -DC + OTR + (-k_5\mu_s^o - k_6\mu_e^o)X \qquad (38d)$$

$$\frac{dG}{dt} = -DG - CTR + (k_7\mu_s^o + k_8\mu_s^r + k_9\mu_e^o)X \qquad (38e)$$

and the additional equation:

$$\frac{dV}{dt} = F = DV \qquad (39)$$

where k_i are the yield coefficients, OTR is the oxygen transfer rate (defined as $OTR = k_L a(C^* - C)$ where $k_L a$ is the mass transfer coefficient and C^* the equilibrium concentration of dissolved oxygen), CTR is the carbon dioxide transfer rate (defined as $CTR = K_V K_L aG$), V is the solution volume in the reactor, F is the input feed rate and D is the dilution rate (defined as D=F/V).

Equations (38) take the matrix form:

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ E \\ C \\ G \end{bmatrix} = \begin{bmatrix} 1 & 1 & 1 \\ -k_1 & -k_2 & 0 \\ 0 & k_3 & -k_4 \\ -k_5 & 0 & -k_6 \\ k_7 & k_8 & k_9 \end{bmatrix}\begin{bmatrix} \mu_s^o \\ \mu_r^s \\ \mu_o^e \end{bmatrix} X - D\begin{bmatrix} X \\ S \\ E \\ C \\ G \end{bmatrix} + \begin{bmatrix} 0 \\ DS_{in} \\ 0 \\ OTR \\ -CTR \end{bmatrix} \qquad (40)$$

which has the structure of the general dynamic model (1) with n=5 state space variables and m=3 reaction rates:

$$\xi^T = \begin{bmatrix} X & S & E & C & G \end{bmatrix} \qquad K^T = \begin{bmatrix} 1 & -k_1 & 0 & -k_5 & k_7 \\ 1 & -k_2 & k_3 & 0 & k_8 \\ 1 & 0 & -k_4 & -k_6 & k_9 \end{bmatrix}$$

$$F^T = \begin{bmatrix} 0 & DS_{in} & 0 & OTR & 0 \end{bmatrix} \quad Q^T = \begin{bmatrix} 0 & 0 & 0 & 0 & CTR \end{bmatrix}$$

As such, the algorithms presented in section 2 can be straightforward applied to the dynamical model (40).

3.2 Estimation problem

There are 3 specific growth rates involved in the dynamical model (40). These are treated as 3 unknown process variables that must be estimated using the OBE or the SODE. As such, the functions $H(\xi)$ and $\rho(\xi)$ in eqn. (3) are defined in the following way:

$$H(\xi) = diag(X) \qquad \rho^T(\xi) = \begin{bmatrix} \mu_s^o & \mu_s^r & \mu_e^o \end{bmatrix}$$

Since there are 3 unknown kinetics, the application of the Luenberger-type asymptotic observer (eqns. 36) to model (40) requires the on-line measurement of 3 state variables. For the same reason, the application of the reduced-order OBE and of the SODE must be based on r=3 state space equations.

Since E, C and G are the state variables more easily accessible on-line, from the practical point of view, it is important to design an estimation scheme based on this set of measurement. Unfortunately, according to the yield coefficient values, E, C and G are linearly dependent, being the corresponding yield matrix ill-conditioned (Pomerleau and Perrier, 1991). Since the inversion of this matrix is required in all the algorithms discussed previously, the numerical implementation would result in extremely sensitive algorithms to numerical errors, thus having performances dramatically degraded.

To solve this problem Pomerleau and Perrier (1991) suggested a reformulation of model (40). This reformulation is based on the division of the complete process model (40) into two "partial" models: (i) the respiro-fermentative partial model (RF) corresponding to the ethanol production state of the process and (ii) the respirative partial model (R) corresponding to the ethanol consumption state of the process.

The fermentative "partial" model (RF) is stated as:

$$
\frac{d}{dt}\begin{bmatrix} X \\ S \\ E \\ C \\ G \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \\ -k_5 & 0 \\ k_7 & k_8 \end{bmatrix}\begin{bmatrix} \mu_s^o \\ \mu_r^s \end{bmatrix} X - D\begin{bmatrix} X \\ S \\ E \\ C \\ G \end{bmatrix} + \begin{bmatrix} 0 \\ DS_{in} \\ 0 \\ OTR \\ -CTR \end{bmatrix}
\tag{41}
$$

and the respirative "partial" model (R) as:

$$
\frac{d}{dt}\begin{bmatrix} X \\ S \\ E \\ C \\ G \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_2 & 0 \\ k_3 & -k_4 \\ 0 & -k_6 \\ k_8 & k_9 \end{bmatrix}\begin{bmatrix} \mu_s^o \\ \mu_o^e \end{bmatrix} X - D\begin{bmatrix} X \\ S \\ E \\ C \\ G \end{bmatrix} + \begin{bmatrix} 0 \\ DS_{in} \\ 0 \\ OTR \\ -CTR \end{bmatrix}
\tag{42}
$$

Notice that they have identical structures with n=5 state space variables but only m=r=2 reaction rates. The only difference between them is reflected on the way the specific growth rates vector and the yield coefficient matrix are stated. For the respiro-fermentative (RF) partial model they are:

$$
\mu_{RF} = \begin{bmatrix} \mu_s^o & \mu_s^r \end{bmatrix}^T \qquad\qquad K_{RF}^T = \begin{bmatrix} 1 & -k_1 & 0 & -k_5 & k_7 \\ 1 & -k_2 & k_3 & 0 & k_8 \end{bmatrix}
$$

and for the respirative partial model (R):

$$
\mu_R = \begin{bmatrix} \mu_s^o & \mu_e^o \end{bmatrix}^T \qquad\qquad K_R^T = \begin{bmatrix} 1 & -k_2 & k_3 & 0 & k_8 \\ 1 & 0 & -k_4 & -k_6 & k_9 \end{bmatrix}
$$

The Luenberger observer, the reduced-order OBE and the SODE must now be applied to both partial models, resulting two "partial" algorithms which must be alternatively used in accordance with the actual process state: ethanol production or ethanol consumption. The success of such an estimation scheme depends upon the detection capability of the correct process state followed by the use of the proper set of equations. The transition between process states can be detected by the transition between positive and negative values of the specific growth rate estimate related to ethanol consumption ($\hat{\mu}_e^o$) or production ($\hat{\mu}_s^r$) (Pomerleau and Perrier, 1991). For example, if the actual process state is ethanol consumption and if the last $\hat{\mu}_e^o$ estimate is negative then the process state has switched from ethanol consumption (R) to ethanol production (RF).

The above mentioned "partial" models have only two specific growth rates involved. This has the very important implication that only two measured state variables are required. Per convenience the variables C and G were chosen. These variables are easily accessible on-line and the numerical problems mentioned previously no longer exist. As

such, the estimation scheme will provide estimates of X, S, E, μ_s^o, μ_e^o and μ_s^r using on-line measurements of C, G, OTR, CTR and F.

3.3 Overall state observation and kinetics estimation scheme

3.3.1 Applying the Luenberger-type asymptotic observer. The application of the Luenberger-type asymptotic observer (eqns. 36) to both partial models (41) and (42) results into two "partial" algorithms with identical structure given by the following set of equations:

$$\frac{d}{dt}\begin{bmatrix} Z_1 \\ Z_2 \\ Z_3 \end{bmatrix} = -D\begin{bmatrix} Z_1 \\ Z_2 \\ Z_3 \end{bmatrix} + \begin{bmatrix} 0 \\ DS_{in} \\ 0 \end{bmatrix} - K_2 K_1^{-1}\begin{bmatrix} OTR \\ -CTR \end{bmatrix} \tag{43a}$$

$$\begin{bmatrix} \hat{X} \\ \hat{S} \\ \hat{E} \end{bmatrix} = \begin{bmatrix} Z_1 \\ Z_2 \\ Z_3 \end{bmatrix} + K_2 K_1^{-1}\begin{bmatrix} C \\ G \end{bmatrix} \tag{43b}$$

The differences between "partial" algorithms are reflected in the way the matrices K_1 and K_2 are defined. These differences are compiled in Table I.

Table I – Differences between "partial" Luenberger-type observers

Respiro-fermentative state(RF)		Respirative state (R)	
$K_1 = K_1^{RF} = \begin{bmatrix} -k_5 & 0 \\ k_7 & k_8 \end{bmatrix}$	(44a)	$K_1 = K_1^{R} = \begin{bmatrix} 0 & -k_6 \\ k_8 & k_9 \end{bmatrix}$	(45a)
$K_2 = K_2^{RF} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_2 \\ k_7 & k_8 \end{bmatrix}$	(44b)	$K_2 = K_2^{RF} = \begin{bmatrix} 1 & 1 \\ -k_2 & 0 \\ k_8 & k_9 \end{bmatrix}$	(45b)

3.3.2 Applying the reduced-order OBE. The application of the reduced-order OBE (eqns. (12)) to both partial models (41) and (42), using the state space equations of C and G, results into two "partial" algorithms with identical structure given by the following set of equations:

$$\psi = K_a^{-1}\begin{bmatrix} C \\ G \end{bmatrix} \tag{46a}$$

$$\frac{d\hat{\psi}}{dt} = X\hat{\mu} - D\psi + K_a^{-1}\begin{bmatrix} OTR \\ -CTR \end{bmatrix} - \Omega(\psi - \hat{\psi}) \tag{46b}$$

$$\frac{d\hat{\mu}}{dt} = X\Gamma(\psi - \hat{\psi}) \tag{46c}$$

The two "partial" OBEs differ on the set of specific growth rates to estimate $\hat{\mu}$, on the yield coefficients matrix K_a, and on the gain matrices Ω and Γ. These differences are compiled in Table II.

<div align="center">Table II – Differences between the "partial" reduced-order OBEs</div>

Respiro-fermentative state (RF)		Respirative state (R)	
$\hat{\mu} = \hat{\mu}_{RF} = \begin{bmatrix} \hat{\mu}_s^o \\ \hat{\mu}_s^r \end{bmatrix}$	(47a)	$\hat{\mu} = \hat{\mu}_R = \begin{bmatrix} \hat{\mu}_s^o \\ \hat{\mu}_e^o \end{bmatrix}$	(48a)
$K_a = K_a^{RF} = \begin{bmatrix} -k_5 & 0 \\ k_7 & k_8 \end{bmatrix}$	(47b)	$K_a = K_a^R = \begin{bmatrix} 0 & -k_6 \\ k_8 & k_9 \end{bmatrix}$	(48b)
$\Omega = \Omega_{RF} = \begin{bmatrix} -\omega_1 & 0 \\ 0 & -\omega_2 \end{bmatrix}$	(47c)	$\Omega = \Omega_R = \begin{bmatrix} -\omega_1 & 0 \\ 0 & -\omega_3 \end{bmatrix}$	(48c)
$\Gamma = \Gamma_{RF} = \begin{bmatrix} \gamma_1 & 0 \\ 0 & \gamma_2 \end{bmatrix}$	(47d)	$\Gamma = \Gamma_R = \begin{bmatrix} \gamma_1 & 0 \\ 0 & \gamma_3 \end{bmatrix}$	(48d)

3.3.3 Applying the second-order dynamics estimator. The application of the SODE (eqns. (13)) to both partial models (41) and (42), using the state space equations of C and G, results into two "partial" algorithms with identical structure given by the following set of equations:

$$\psi = K_a^{-1}\begin{bmatrix} C \\ G \end{bmatrix} \tag{49a}$$

$$\frac{d\hat{\psi}}{dt} = X\hat{\mu} - D\psi + K_a^{-1}\begin{bmatrix} OTR \\ -CTR \end{bmatrix} - \Omega(t)(\psi - \hat{\psi}) \tag{49b}$$

$$\frac{d\hat{\mu}}{dt} = X^{-1}\Gamma(\psi - \hat{\psi}) \tag{49c}$$

As in the case of the reduced-order OBE, the two "partial" SODEs differ on the set of specific growth rates to estimate $\hat{\mu}$, on the yield coefficients matrix K_a, and on the gain matrices $\Omega(t)$ and Γ. These differences are compiled in Table III.

Table III – Differences between the "partial" SODEs

Respiro-fermentative state (RF)		Respirative state (R)	
$\hat{\mu} = \hat{\mu}_{RF} = \begin{bmatrix} \hat{\mu}_s^o \\ \hat{\mu}_s^r \end{bmatrix}$	(50a)	$\hat{\mu} = \hat{\mu}_R = \begin{bmatrix} \hat{\mu}_s^o \\ \hat{\mu}_e^o \end{bmatrix}$	(51a)
$K_a = K_a^{RF} = \begin{bmatrix} -k_5 & 0 \\ k_7 & k_8 \end{bmatrix}$	(50b)	$K_a = K_a^{R} = \begin{bmatrix} 0 & -k_6 \\ k_8 & k_9 \end{bmatrix}$	(51b)
$\Omega(t) = \Omega_{RF}(t) =$ $\begin{bmatrix} -\dfrac{2\zeta_1}{\tau_1} - \phi(X) & 0 \\ 0 & -\dfrac{2\zeta_2}{\tau_2} - \phi(X) \end{bmatrix}$	(50c)	$\Omega(t) = \Omega_{R}(t) =$ $\begin{bmatrix} -\dfrac{2\zeta_1}{\tau_1} - \phi(X) & 0 \\ 0 & -\dfrac{2\zeta_3}{\tau_3} - \phi(X) \end{bmatrix}$	(51c)
$\Gamma = \Gamma_{RF} = \begin{bmatrix} \dfrac{1}{\tau_1^2} & 0 \\ 0 & \dfrac{1}{\tau_2^2} \end{bmatrix}$	(50d)	$\Gamma = \Gamma_{R} = \begin{bmatrix} \dfrac{1}{\tau_1^2} & 0 \\ 0 & \dfrac{1}{\tau_3^2} \end{bmatrix}$	(51d)

Notice that in eqns. (51c) and (50c), $\phi(X)$ is given by:

$$\phi(X) = \frac{X_{t+1} - X_t}{X_t T} \tag{52}$$

where T refers to the integration step, X_{t+1} and X_t to biomass concentration at the time instances t+1 and t respectively.

3.3.4 Switching mechanism between "partial" algorithms. The successful application of the "partial" algorithms mentioned above requires an on-line detection strategy of the current process state: ethanol production (RF) or ethanol consumption (R). Pomerleau and Perrier (1991) concluded that this detection mechanism could be based on the transition between positive and negative values of the specific growth rate estimate related to ethanol (consumption-$\hat{\mu}_e^o$ or production-$\hat{\mu}_s^r$). For example, if the actual process

state is ethanol consumption and if the last $\hat{\mu}_e^o$ estimate is negative then the process state has switched from ethanol consumption (R) to ethanol production (RF).

As such, the overall estimation scheme will consists on three steps:

1) Integration of the "partial" Luenberger-type observer (eqns. (43)) correspondent to the actual process state.
2) Integration of the "partial" reduced-order OBE (eqns. (46)) or the "partial" SODE (eqns. (49)) correspondent to the actual process state.
3) Check if transition between process states has occurred by checking the signal of specific growth rate estimate related to ethanol.

From the practical point of view, the transition between "partial" algorithms can be realised just by switching between eqns. (44a-b)/(45a-b) and, between (47a-c)/(48a-c) when the reduced-order OBE is used or between (50a-c)/(51a-c) when the SODE is used.

4 RESULTS AND DISCUSSION

The performance of the estimation algorithms will be analysed at aid of a simulation experiment. The process dynamical model (40) was implemented on a process simulator (Pimenta *et al.*, 1993) assuming the kinetic model proposed by Sonnleitner and Käppeli (1986) (the values of the kinetic parameters used are listed in Table IV). The simulation experiment was made under the following initial conditions:

X(0)=1.0 g/L, S(0)=0.02 g/L, E(0)=0.15 g/L, C(0)=0.0066 g/L, G(0)=0.008 g/L, V(0)=3.5 L

Table IV - Kinetic parameters(taken from Sonnleitner and Käppeli (1986))

Parameter	Value
$q_{s,max}$	3.50 g glucose/(g biomass·h)
$q_{c,max}$	0.256 g O_2/(g biomass·h)
$q_{e,max}$	0.236 g ethanol/(g biomass·h)
K_e	0.10 g·L^{-1}
K_i	0.10 g·L^{-1}
K_s	0.2 g·L^{-1}
K_c	0.0001 g·L^{-1}

The value for $K_L a$, K_V and C^* were assumed 100hr^{-1}, 0.2 and 0.007 g/l respectively. The values of the yield coefficients are listed in Table V.

Table IV - Yield coefficients (taken from Sonnleitner and Käppeli (1986))

Coefficient	(Coefficient)$^{-1}$	Value
Y_o	k_1	0.49 g biomass/g glucose
Y_r	k_2	0.05 g biomass/g glucose
Y_{re}	k_3	0.10 g biomass/g ethanol
Y_e	k_4	0.72 g biomass/g ethanol
Y_c	k_5	1.20 g biomass/g O_2
Y_{ce}	k_6	0.64 g biomass/g O_2
Y_{go}	k_7	0.81 g biomass/g CO_2
Y_{gr}	k_8	0.11 g biomass/g CO_2
Y_{ge}	k_9	1.11 g biomass/g CO_2

A fermentation run of 18 hours is assumed. In Fig. 1 the used feed rate profile and the corresponding broth volume evolution are shown. The profiles of the gaseous outflow rates, of the state variables, and the specific growth rates are shown in Figs. 2, 3 and 4 respectively.
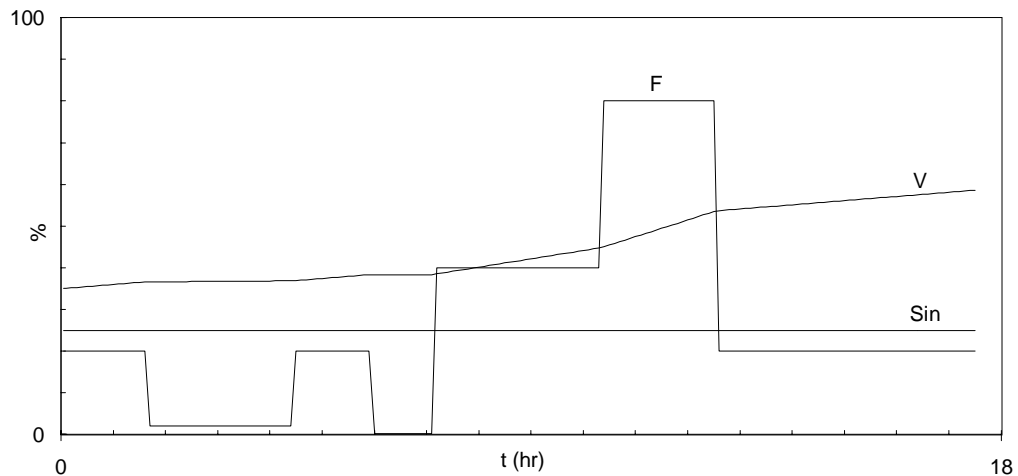
Fig. 1. Input feed rate F(0.0 - 0.5 L/h), glucose concentration on the feed Sin (250 g/L) and volume V (0.0 - 10.0 L)
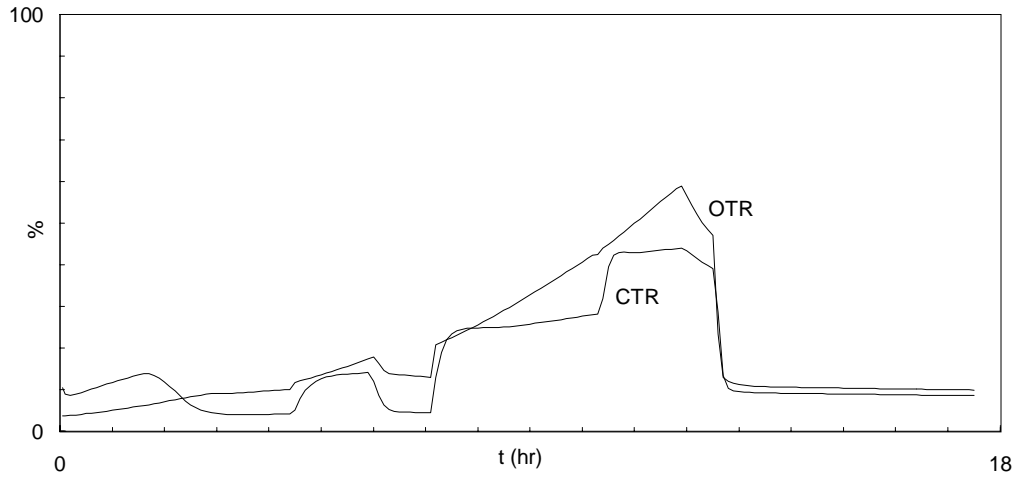
Fig. 2. Gaseous transfer rates: Oxygen transfer rate OTR (0.0 – 7.0 g L^{-1}h^{-1}) and carbon dioxide transfer rate CTR (0.0 – 12.0 g L^{-1}h^{-1})
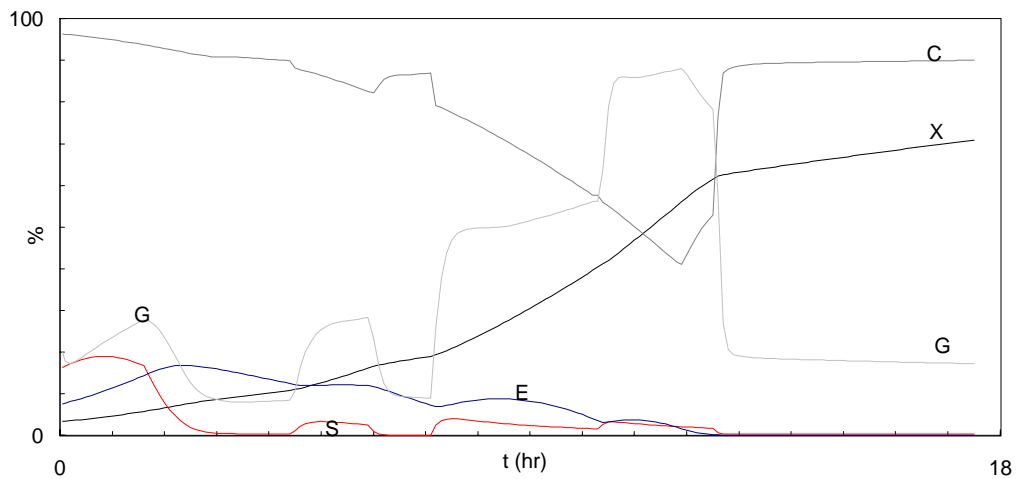

Fig. 3. State space variables: biomass X (0.0 – 30.0 g/L), glucose S (0.0 - 1.25 g/L), ethanol E (0.0 - 2.0 g/L), oxygen C (0.0 - 0.007 g/L) and carbon dioxide G (0.0 - 0.03 g/L).

With the input feed rate of Fig. 1 and with the initial conditions mentioned above the switch between respiro-fermentative and respirative catabolic states occurred 6 times. Fig. 4 includes at the top a ruler distinguishing the two different process states: respiro-fermentative (RF) with ethanol production, and respirative (R) with ethanol consumption. The points when the process state switch occurs are marked with the letters a, b, c, d, e, f, and g.
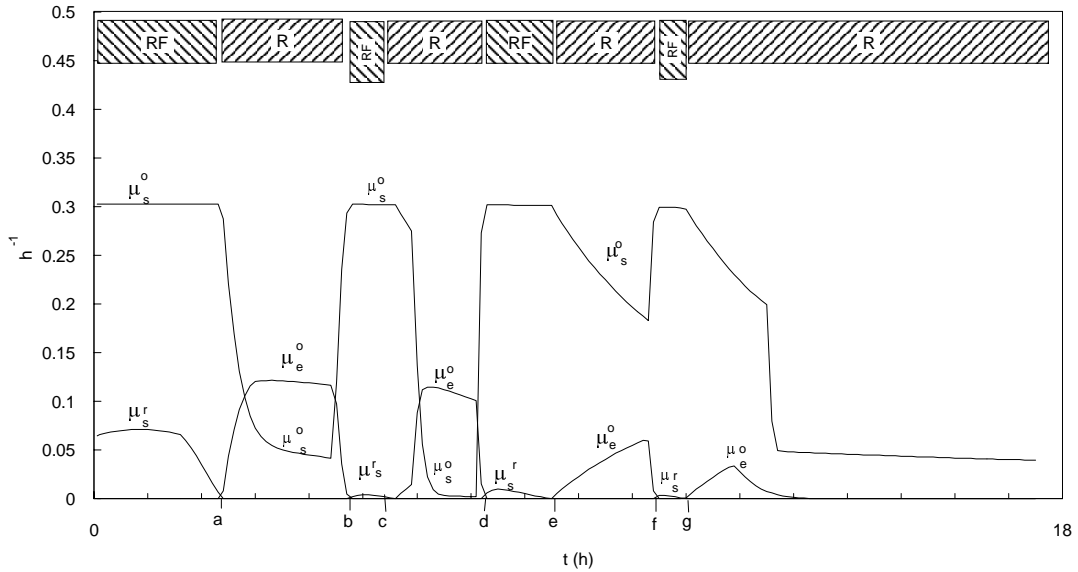
Fig. 4. Specific growth rates profiles using the kinetic model proposed by Sonnleitner and Käppeli (1986), with the kinetic parameters listed in Table IV.

This simulation experiment supplied the estimation algorithm with the relevant measured variables: C, G, OTR, CTR, Sin, V and F at a sampling rate of 6 minutes. The behaviour of the estimation scheme developed in the previous section will now be analysed at aid of this simulation experiment. As such, the curves shown in Fig.4 are taken as the "true" specific growth rates profiles. The variables C, G, OTR, CTR, and F in Figs. 1, 2 and 3 are considered as process on-line measurements, being supplied to the estimation algorithms at a sampling rate of 6 minutes.

The following figures illustrate the use of the reduced-order OBE and of the SODE. Each of them includes a ruler similar to the one of Fig. 4 distinguishing the two different process states: respiro-fermentative (RF) with ethanol production, and respirative (R) with ethanol consumption. The points when the switch between process states occur are marked with the letters a, b, c, d, e, f and g. The "true" specific growth rates of Fig. 4 are represented by the dotted lines while the respective estimates are represented by the full lines. The accuracy of the estimates can be assessed by the ITAE error index (ITAE - integral of time-weighted absolute errors) given in the legend for the three specific growth rates.

Figs. 5, 6 and 7 show the results produced by the reduced-order OBE for three different tunings (defined heuristically). The equations were integrated with a robust variable step integration algorithm (4th/5th order Runge-Kutta type embedded scheme due to Butcher) employing along the integration linear estimates of the relevant sampled variables.
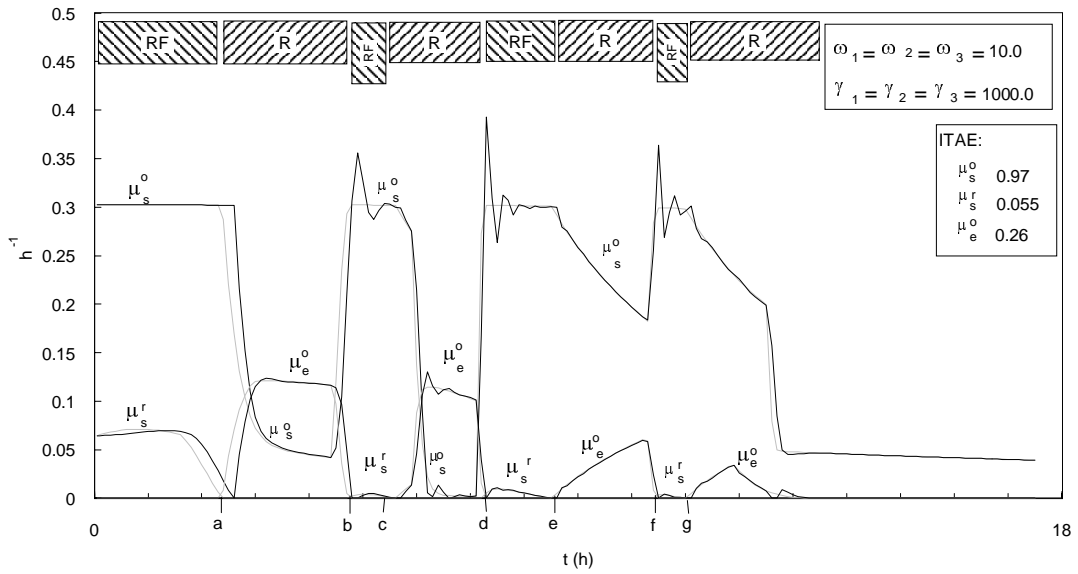
Fig. 5. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the reduced-order OBE integrated with an 4th/5th order variable step integration routine.
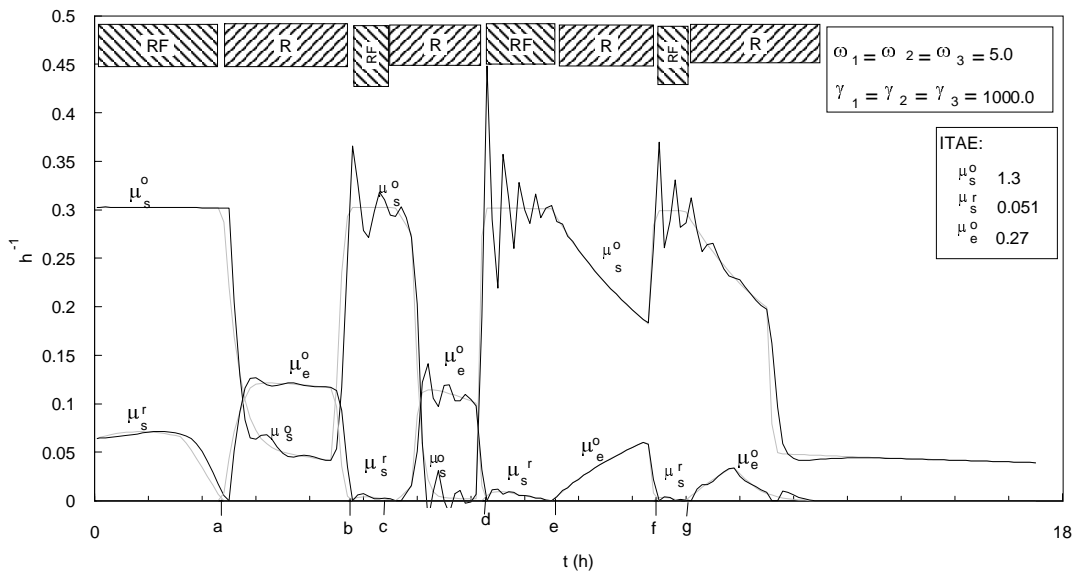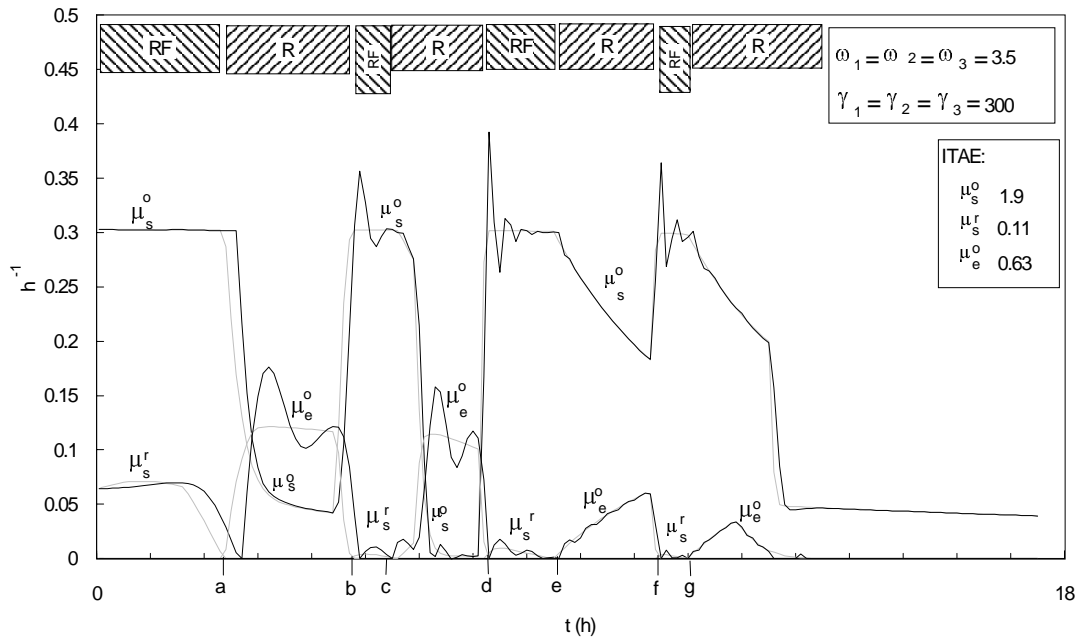

Fig. 6. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the reduced-order OBE integrated with an 4th/5th order variable step integration routine.

Fig. 7. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the reduced-order OBE integrated with an 4th/5th order variable step integration routine.

A careful analysis of the plots reveals that the dynamic of convergence is time-varying, i. e., the response becomes increasingly faster and oscillatory as the run approaches the end.

The results yield by the SODE are depicted in Figs (8-14). The equations were integrated with a robust variable step integration algorithm similar to the one used for the reduced-order OBE. The influence of ζ can be assessed from the plots in Figs (8-11) where τ is kept constant at 0.15 hours while ζ assumes the values 0.25, 0.5, 1.0 and 1.25 respectively. The influence of τ can be assessed from the plots in Figs (10) and (11-14) where ζ is kept constant at 1.0 while τ assumes 0.15, 0.1, 0.05 and 0.01 hours respectively.
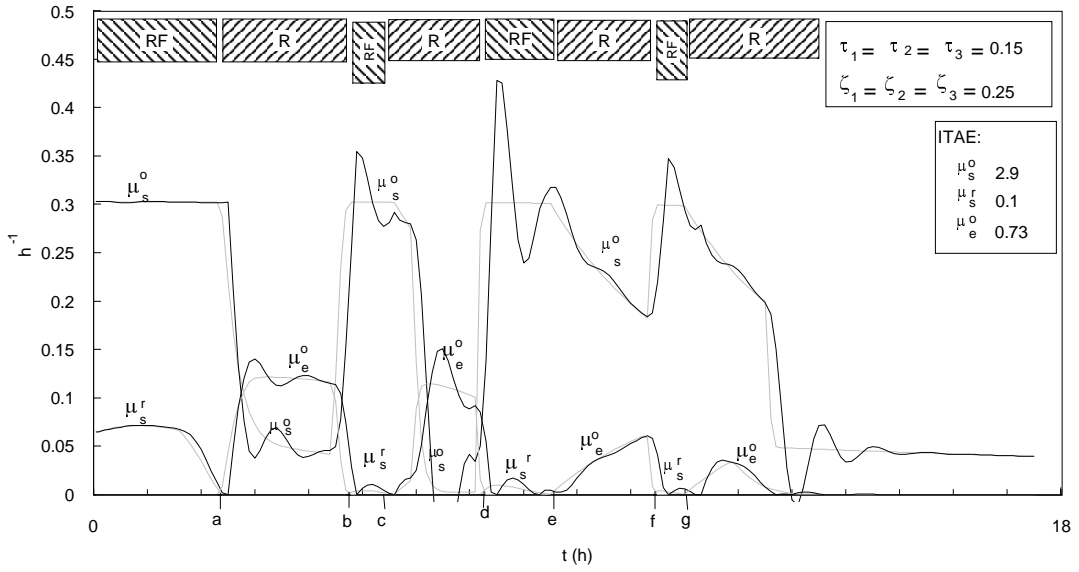
Fig. 8. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE integrated with an 4th/5th order variable step integration routine.
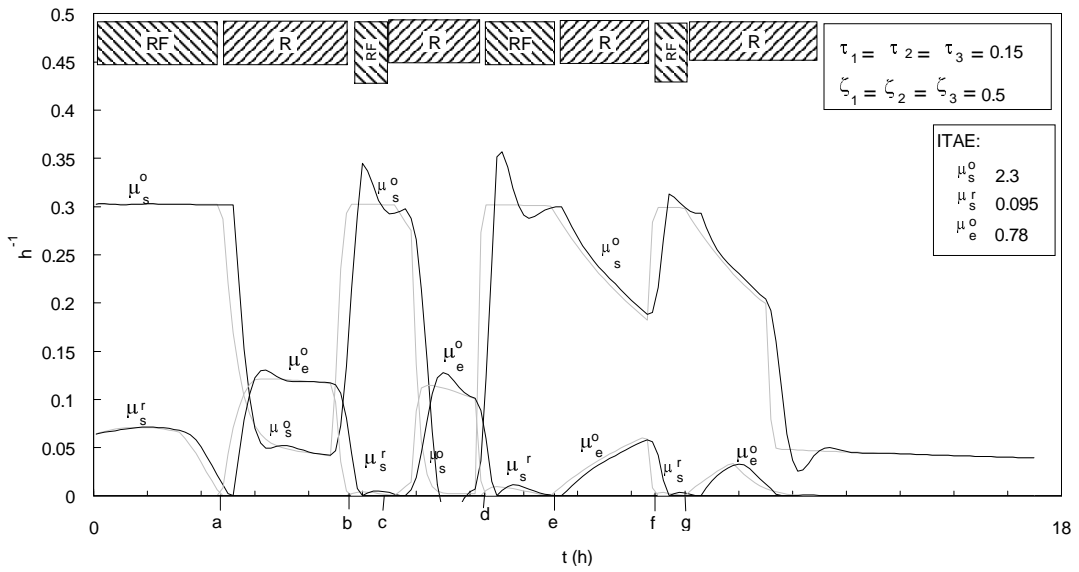

Fig. 9. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE integrated with an 4th/5th order variable step integration routine.
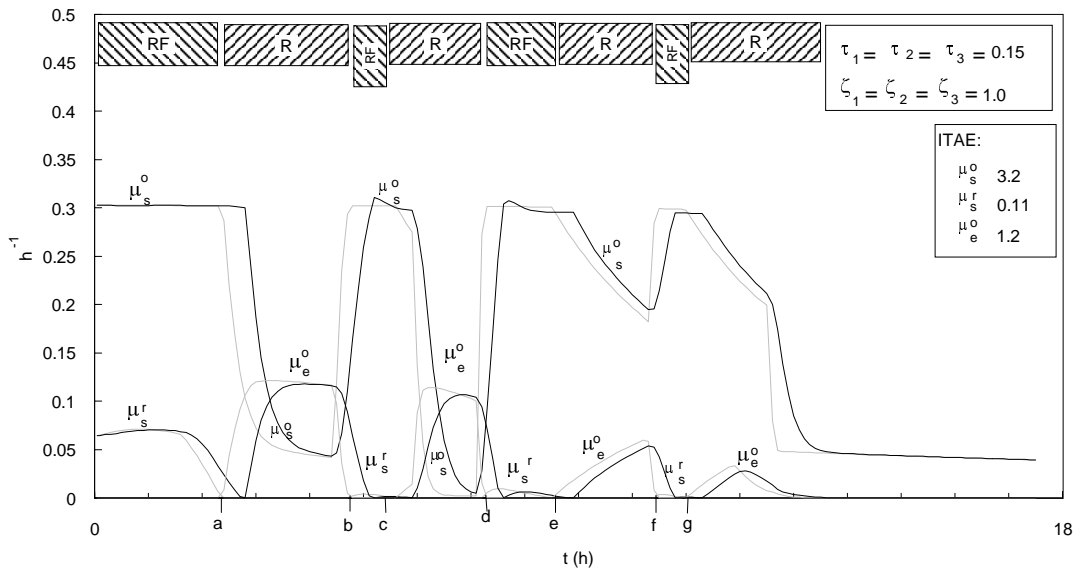
Fig. 10. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE integrated with an 4th/5th order variable step integration routine.

Fig. 11. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE integrated with an 4th/5th order variable step integration routine.

Fig. 12. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE integrated with an 4th/5th order variable step integration routine.

Fig. 13. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE integrated with an 4th/5th order variable step integration routine.
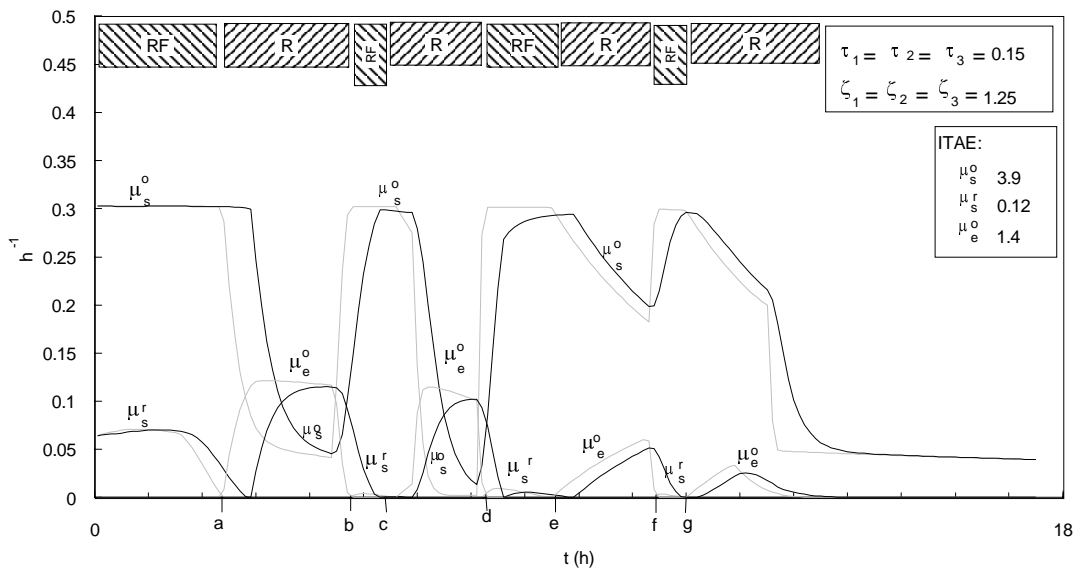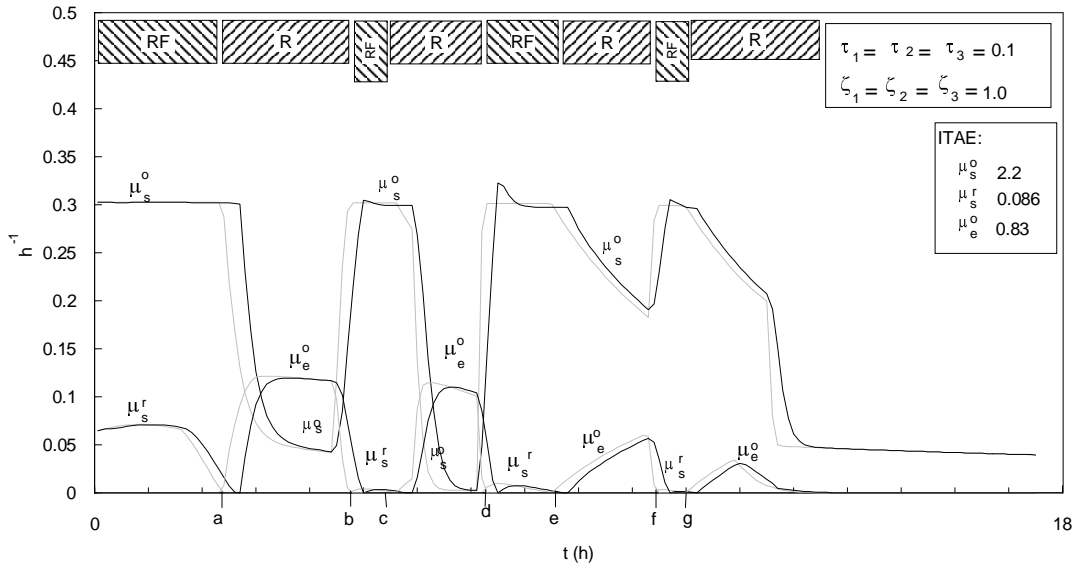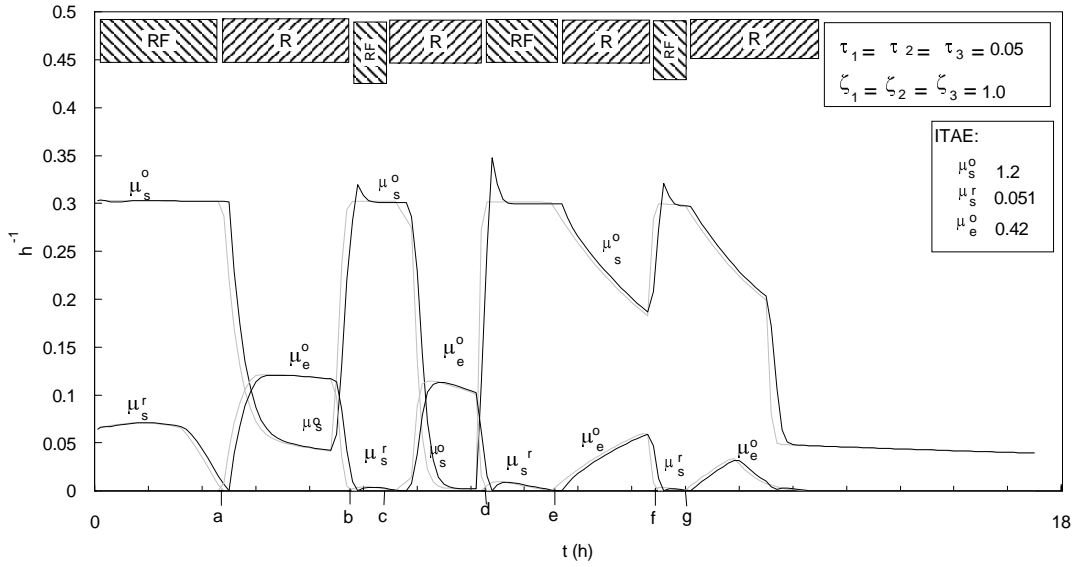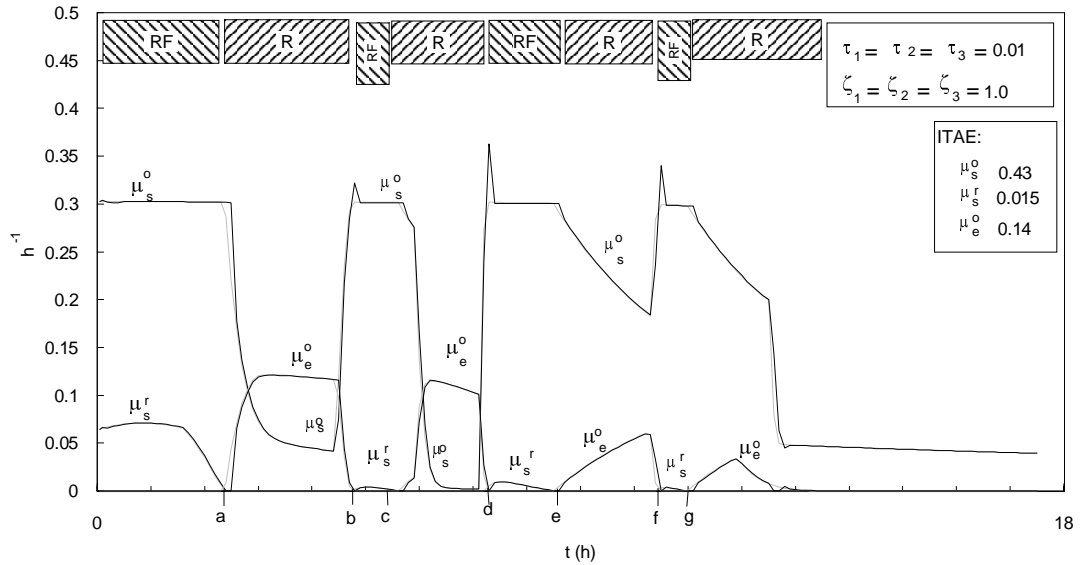
Fig. 14. Specific growth rates estimated (full lines) and "true" (dotted lines) given by the SODE integrated with an 4th/5th order variable step integration routine.

As given by the plots in Figs. (8-14) the characteristics of the dynamics of convergence of the estimated values to the true values appears to be in agreement with a typical second-order dynamical response. It is shown that decreasing τ produces faster responses, while decreasing ζ produces more oscillatory responses. Furthermore, from the plots in Figs (8-11) it can be concluded that $\zeta=1$ constitutes the frontier between oscillatory and non oscillatory behaviour.

In section (2.2.3) the numerical implementation of the SODE was discussed. It was shown that a forward Euler discretisation of the continuous equations poses stability problems. In particular, three relations (eqns. (30), (32) and (33)) were derived which define stable intervals for the integration step T in relation to a specific tuning. The following Figs. attempt to illustrate the behaviour of the SODE with an Euler discretisation, when the stability limits are disobeyed. The integration step and the sampling time were assumed to be 6 minutes.

The results in Figs (15-17) were obtained with $\zeta=0.75$. As given by eqn. (30), if T=0.1 h then τ must be larger then 0.07. The results obtained with $\tau=0.07$, $\tau=0.075$ and $\tau=0.085$ hours are shown in Figs. (15), (16) and (17) respectively.

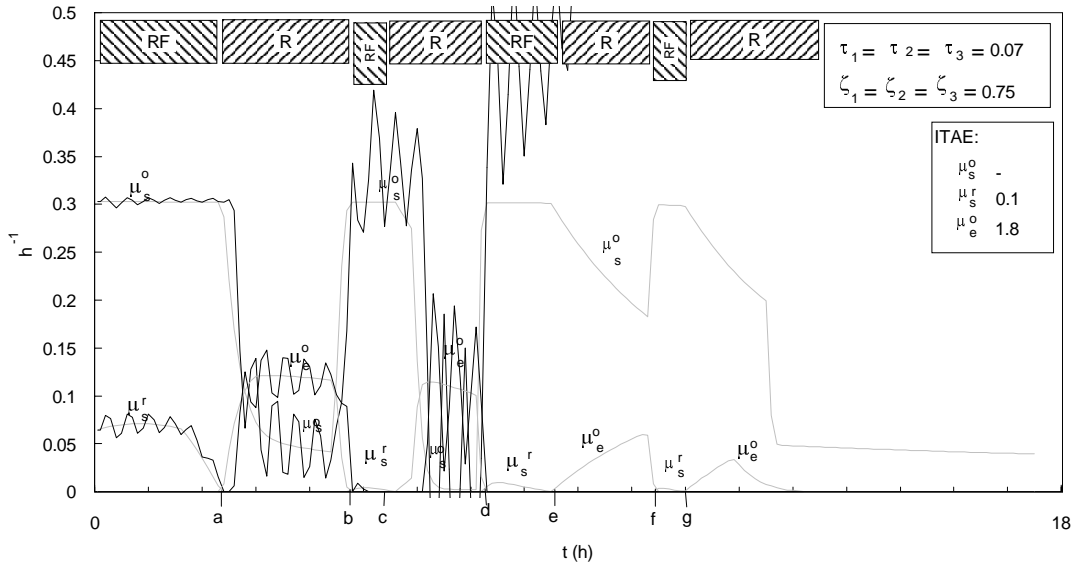Fig. 15. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE using Euler discretisation.
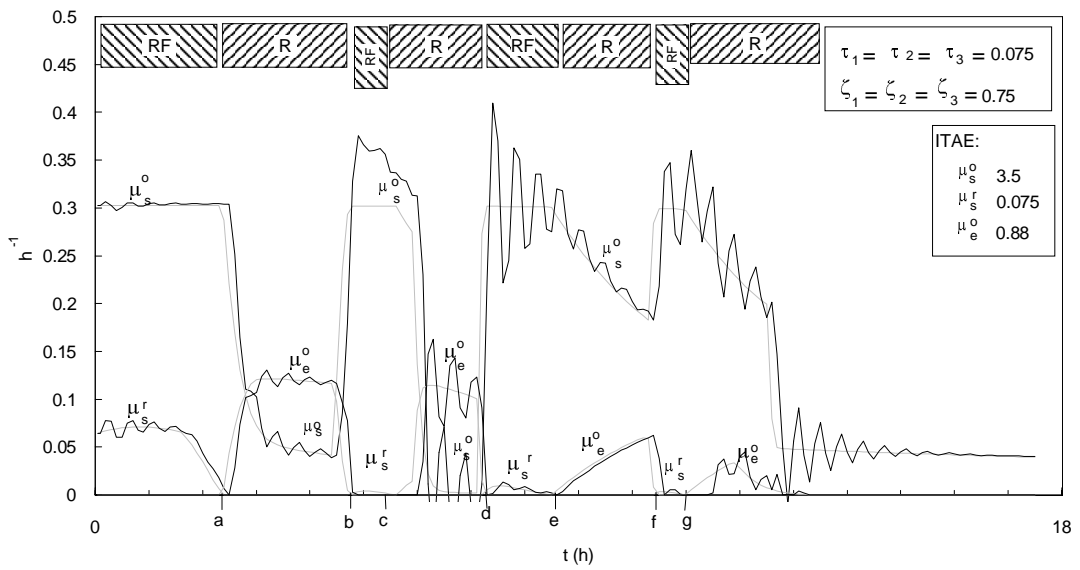
Fig. 16. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE with Euler discretisation.

Fig. 17. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE with Euler discretisation.

With $\tau=0.07$ the estimated curves diverge, and the algorithm is thus unstable. Increasing τ to 0.075 the divergence stopped being observed. Nevertheless, persistent oscillations are exhibited suggesting that the estimator operates near the stability limit. For $\tau=0.085$ a normal output is obtained.

The results in Figs (18-20) were obtained with $\zeta=1.0$. As given by eqn. (32), if T=0.1hr then τ must be larger then 0.1. The results obtained with $\tau=0.095$, $\tau=0.1$ and $\tau=0.105$ are shown in Figs. (18), (19) and (20) respectively.

With $\tau=0.095$ the algorithm reveals itself unstable. Increasing τ to 0.1 the divergence stopped being observed. Nevertheless, the response exhibits occasionally overshoot which is not characteristic of a second-order response with $\zeta=1$. This suggests that the estimator operates near the stability limit. For $\tau=0.105$ the overshoot is reduced suggesting that the estimator operates farther from the stability limit.

Fig. 18. Specific growth rates estimates (full lines) and true (dotted lines) given by the SODE with Euler discretisation.

Fig. 19. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE with Euler discretisation.
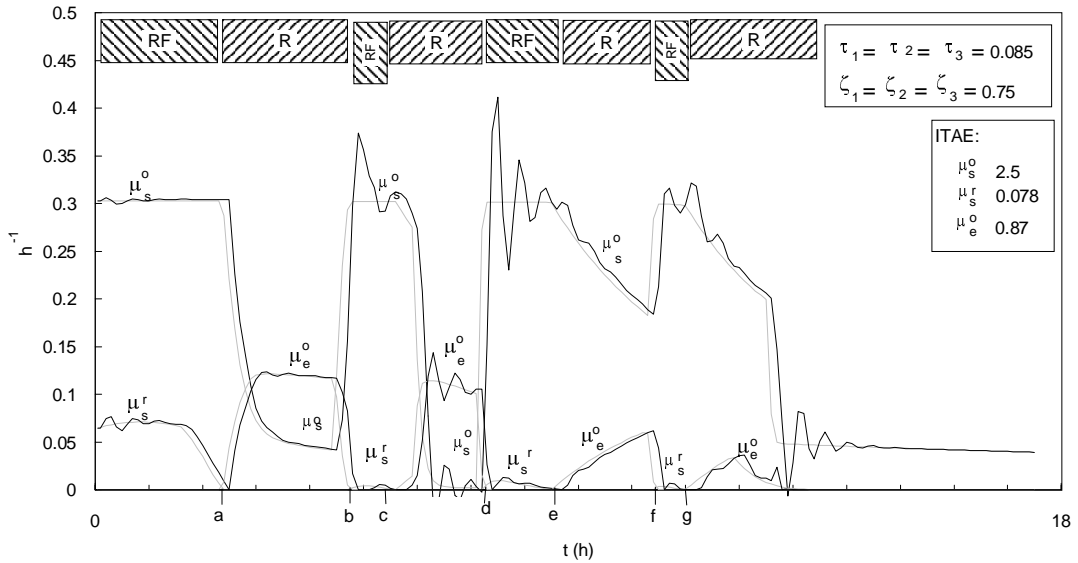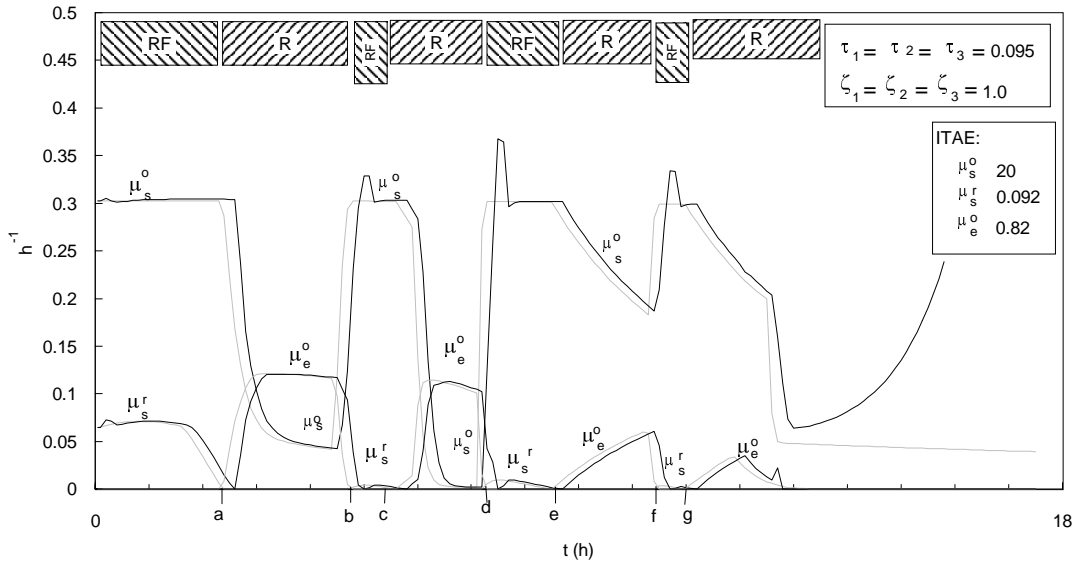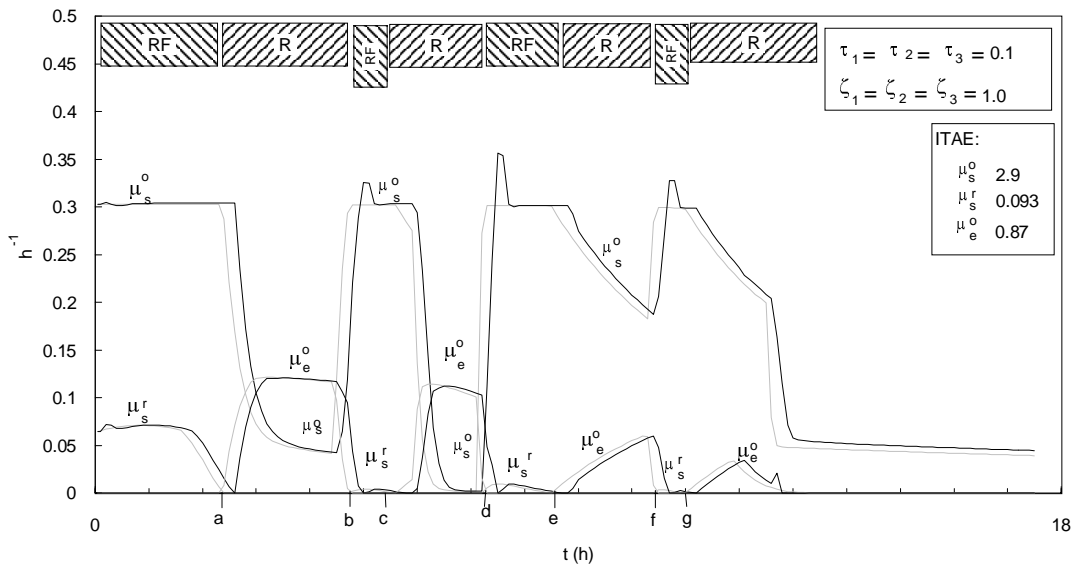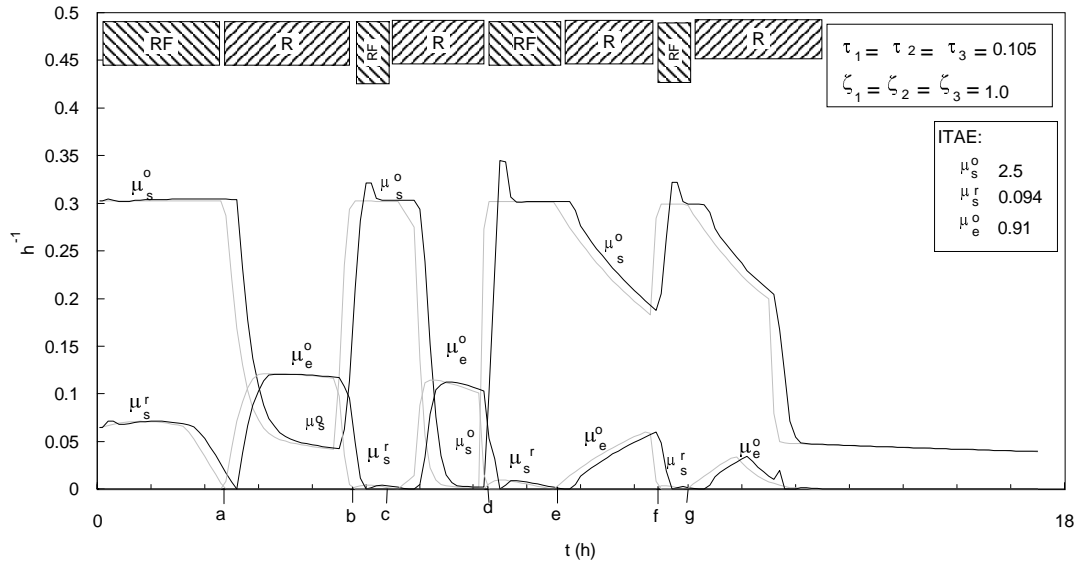
Fig. 20. Specific growth rates estimates (full lines) and "true" (dotted lines) given by the SODE with Euler discretisation.

APPENDIX: Proof of Uniform Asymptotic Stability of the SODE Error System

Let's first consider the following reformulated error system and concentrate our attention to the unforced system :

$$\frac{dy}{dt} = -a(t)y + \tilde{\rho} \tag{A-1a}$$

$$\frac{d\tilde{\rho}}{dt} = -\gamma y + \frac{d\rho}{dt} \tag{A-1b}$$

which can be obtained from system (15) by considering the transformation $y = \tilde{\psi}/h$ (to simplify the present analysis the index "i" will be omitted), where

$$a(t) = \omega(t) + h^{-1}\frac{dh}{dt} \tag{A-2}$$

Choosing the following candidate Lyapunov function:

$$V(y, \tilde{\rho}) = \gamma y^2 + \tilde{\rho}^2 \tag{A3}$$

whose time derivative along the solution of (A-1) is given by:

$$\frac{dV}{dt} = -2a(t)y^2 = -E^T Q(t)E \tag{A-4}$$

where

$$E^T = \begin{bmatrix} y & \tilde{\rho} \end{bmatrix} \qquad Q(t) = \begin{bmatrix} a(t) & 0 \\ 0 & 0 \end{bmatrix}$$

then it follows that if $a(t) \geq 0 \quad \forall_{t \geq t_0}$ then $Q(t)$ is positive semi-definite. Hence the equilibrium state $E=0$ is uniformly stable (Narendra and Annaswamy, 1989). Supposing that conditions C1 thorough C3 hold, the conditions under which $a(t) \geq 0$ $\forall_{t \geq t_0}$ are stated as:

C5. $h(\xi)$ is a differentiable function of ξ, which means that $|\dot{h}|$ is bounded

C6. $h(\xi)$ is bounded as follows:
$$0 < h_{min} \leq h_i(\xi) \leq h_{max} \quad \forall_{t \geq t_0}$$

C7. $\omega(t) \geq -\frac{1}{h}\frac{dh}{dt} \quad \forall_{t \geq t_0}$

Still, since $Q(t)$ is positive semi-definite and time-varying, it can not be concluded that system (A-1) is uniformly asymptotically stable.

In the lines bellow, the exponential stability of (A-1) is proofed. A qualitative outline of the proof can be given in two steps: *i)* in system (A-1) $y(t)$ has to assume a large value at

some instance in every *interval [t, t+T_O]*, *ii)* since $\dot{V}$ is as given by eqn. (A-3), this implies that *V(t)* decreases over every interval of length T_O which assures uniform asymptotic stability.

Proof. Suppose that

$$\left| y_i(t) \right| < \alpha \left\| E_i(t) \right\| \quad \forall t \in \left[t_o, t_o + T_o \right]$$

where $\alpha \in [0,1]$. Integrating (A-1a) over the time interval $\left[t_2, t_2 + \delta_0 \right] \subset \left[t_0, t_0 + T_0 \right]$, it follows that

$$\left| y(t_2 + \delta_0) \right| + \left| y(t_2) \right| \geq \int_{t_2}^{t_2 + \delta_0} \left| \tilde{\rho}(\tau) \right| d\tau - a_{max} \int_{t_2}^{t_2 + \delta_0} \left| y(\tau) \right| d\tau \tag{A-5}$$

where a_{max} is the maximum value of $\left| a(t) \right|$. Therefore

$$\left| y(t_2 + \delta_0) \right| \geq \int_{t_2}^{t_2 + \delta_0} \left| \tilde{\rho}(\tau) \right| d\tau - (\delta_0 a_{max} + 1) \sup_{\tau \in [t_2, t_2 + \delta_0]} \left| y(\tau) \right| \tag{A-6}$$

since $\left| y(t_2) \right|$ is always less then $\sup \left| y(\tau) \right|$ in $\tau \in \left[t_2, t_2 + \delta_0 \right]$. On the other hand

$$\int_{t_2}^{t_2 + \delta_0} \left| \tilde{\rho}(\tau) \right| d\tau \geq \int_{t_2}^{t_2 + \delta_0} \left| \tilde{\rho}(t_2) \right| d\tau - \int_{t_2}^{t_2 + \delta_0} \left| \tilde{\rho}(t_2) - \tilde{\rho}(\tau) \right| d\tau$$

$$\geq \delta_0 \left| \tilde{\rho}(t_2) \right| - \delta_0 \sup_{\tau \in [t_2, t_2 + \delta_0]} \left| \tilde{\rho}(t_2) - \tilde{\rho}(\tau) \right|$$

$$\geq \delta_0 \left| \tilde{\rho}(t_2) \right| - \delta_0 \int_{t_2}^{t_2 + \delta_0} \left| \dot{\tilde{\rho}} \right| d\tau \tag{A-7}$$

since the distance between to points $\tilde{\rho}(t_2)$ and $\tilde{\rho}(\tau)$ is always less then the arc length $\int_{t_2}^{t_2 + \delta_0} \left| \dot{\tilde{\rho}} \right| d\tau$. Hence, evaluating $\dot{\tilde{\rho}}$ from (A-1b), eqn. (A-7) becomes

$$\int_{t_2}^{t_2 + \delta_0} \left| \tilde{\rho}(\tau) \right| d\tau \geq \delta_0 \left| \tilde{\rho}(t_2) \right| - b \sup_{\tau \in [t_2, t_2 + \delta_0]} \left| y(\tau) \right| \tag{A-8}$$

where $b = \gamma \delta_0^2 + a_{max} \delta_0 + 1$. From the initial supposition regarding $\left| y(t) \right|$, it follows that $\sup \left| y(\tau) \right|$ in $\tau \in \left[t_2, t_2 + \delta_0 \right]$ is always less then $\alpha \left\| E(t_2 + \delta_0) \right\|$, and also $\left| \tilde{\rho}(t_2) \right|$ is always larger then $\sqrt{1 - \alpha^2} \left\| E(t_2) \right\|$. Since

$$\frac{d \left\| E(t) \right\|}{dt} \leq \frac{-a(t) y^2}{\min(\gamma, 1)} \leq 0 \quad \forall t \geq t_0$$

then $\left\|E(t2)\right\| \geq \left\|E(t_2 + \delta_0)\right\|$. Hence eqn. (A-8) becomes

$$\left|y(t_2 + \delta_0)\right| \geq \left(\delta_0 \sqrt{1 - \alpha^2} - b\alpha\right)\left\|E(t_2 + \delta_0)\right\| \tag{A-9}$$

Choosing

$$\alpha^2 = \frac{\delta_0^2}{\delta_0^2 + (1+b)^2} \tag{A-10}$$

then eqn. (A-9) becomes

$$\left|y(t_2 + \delta_0)\right| \geq \alpha\left\|E(t_2 + \delta_0)\right\| \tag{A-11}$$

which is a contradiction of the initial assumption regarding $\left|y(t)\right|$. Point *i)* is therefore proofed.

Let's now integrate eqn. (A-1b) over a time interval $\left[t_1, t_1 + T\right] \subset \left[t_0, t_0 + T_0\right]$

$$V(t_1) - V(t_1 + T) \geq 2a_{\max} \int\limits_{t_1}^{t_1+T} \left|y(\tau)\right|^2 d\tau \tag{A-12}$$

and by the Cauchy-Schwarz inequality

$$V(t_1) - V(t_1 + T) \geq \frac{2a_{\max}}{T}\left(\int\limits_{t_1}^{t_1+T} \left|y(\tau)\right| d\tau\right)^2 \tag{A-13}$$

Further, by considering that

$$\int\limits_{t_1}^{t_1+T} \left|y(\tau)\right| d\tau \geq \int\limits_{t_1}^{t_1+T} \left|y(t_1)\right| d\tau - \int\limits_{t_2}^{t_2+\delta_0} \left|\dot{y}\right| d\tau$$
$$\geq T\left|y(t_1)\right| - T^2 d\left\|E(t_1)\right\| \tag{A-14}$$

where $d = a_{\max} + 1$, then choosing $t_1 = t_2 + \delta_0$ we have that

$$\int\limits_{t_1}^{t_1+T} \left|y(\tau)\right| d\tau \geq T\left(\alpha - Td\right)\left\|E(t_1)\right\| \tag{A-15}$$

Hence eqn. (A-13) becomes

$$V(t_1) - V(t_1 + T) \geq 2\frac{a_{\max}}{d} Td\left(\alpha - Td\right)^2 \left\|E(t_1)\right\|^2 \tag{A-16}$$

being T less than α/d. Since $\|E(t)\|^2$ is always larger then $V(t)/\max(\gamma,1)$, then choosing $T = \min(t_0 + T_0 - t_1, \alpha/d)$ we conclude that

$$V(t_0 + T_0) \leq V(t_1 + T) \leq (1-\beta)V(t_1) \leq (1-\beta)V(t_0) \tag{A-17}$$

where

$$\beta = \frac{2a_{max}}{\max(\gamma,1)d} Td(\alpha - Td)^2 \tag{A-18}$$

Since a_{max}/d is always less then *1* and *T=α/(3d)* is a maximum point of the function $f(T) = Td(\alpha - Td)^2$, we conclude that

$$\beta \in \left[0, \frac{8\alpha^3}{27\max(\gamma,1)}\right] \subset [0,1] \tag{A-19}$$

and, hence, the final conclusion can be taken that the estimator (A-1) is uniformly asymptotically stable.

NOMENCLATURE

C	oxygen concentration
CTR	carbon dioxide transfer rate
D	dilution rate
E	ethanol concentration
F	mass feed rate vector
G	carbon dioxide concentration
$H(\xi)$	$(m \times r)$ matrix of known functions of the state
K	yield coefficients matrix
k_i	yield coefficients
OTR	Oxygen transfer rate
Q	rate of mass removel in gaseous form vector
S	glucose concentration
S_{in}	glucose concentration in the feed
T	sampling period
X	biomass concentration

Greek letters

φ	reaction rates vector
$\hat{\xi}$	estimated state vector
μ_s^r	specific growth rate for the fermentative growth on glucose pathway
μ_e^o	specific growth rate for the respiratory growth on ethanol pathway
μ_s^o	specific growth rate for the respiratory growth on glucose pathway
$\hat{\xi}_2$	estimated vector of nonmeasured state variables
$\hat{\mu}$	vector of estimated specific growth rates
Ω, Γ	gain matrices
τ_i	natural period of oscillation
μ	specific growth rates vector
$\hat{\rho}$	vector of estimatives of $\rho(t)$
$\rho(t)$	vector of completely unknown time-varying parameters
ω_i, γ_i	diagonal elements of Ω and Γ
ζ_i	damping coefficient
ξ	state space vector
ξ_1	measured state space vector
ξ_2	non-measured state space vector

Mathematical notations

sup	supremun
min	minimum
max	maximum
diag{.}	diagonal matrix

REFERENCES

Bastin, G. and D. Dochain (1990) "On-Line Estimation and Adaptive Control of Bioreactors," Elsevier, Amsterdam.

Pomerleau, Y. and M. Perrier (1990) "Estimation of Multiple Specific Growth Rates in Bioprocesses," *AIChE Journal*, **36(2)**, pp. 207-215.

Pomerleau, Y. (1990), "Modélisation et contrôle d'un procédé fed-batch de culture des levures à pain", Ph. D. Thesis, Ecole Polytechnique de Montréal, Canada.

Sonnleitner, B. and O. Käppeli (1986) "Growth of *Saccharomyces cerevisiae* is controlled by its Limited Respiratory Capacity: Formulation and Verification of a Hypothesis," *Biotech. Bioeng.*, **28**, pp. 927-937.

Luenberger, D.G. (1971) "An introdution to observers," *IEEE Trans. Automatic Control*, **AC-16**, pp. 596-603.

Feyo de Azevedo, S., P. Pimenta, F. Oliveira, E. Ferreira (1992) "Studies on On-Line State and Parameter Estimation through a Real-Time Process Simulator," in Karim, M. N. and Stephanopoulos, J. (Eds.), Modeling and Control of Biotschnical Processes, IFAC Symp. Series 10, PP 453-458, Pergamon Press N.Y.

Narendra, K.S. and A.M. Annaswany (1989) "Stable Adaptive Systems," Prentice Hall, Englewood Cliffs

Chapter 7

Conclusions

The costs of developing mathematical mechanistic models for bioprocesses improvement are often too high and the benefits too low. There are several reasons for this. The main reason is related to the intrinsic complexity of biological systems. Unfortunately many bioprocess mechanisms are not yet well understood. This is particularly critical in what the microorganisms growth kinetics concerns. In general, mathematical descriptions of growth kinetics assume hard simplifications based on unstructured and non-segregated cell models. These models are often not accurate enough at describing the underlying mechanisms. An accuracy increase can only be achieved by activating more knowledge. This is precisely the critical issue. Acquisition of mechanistic knowledge is slow and expensive. As such, the bottleneck in developing a mechanistic mathematical model with the required accuracy lies in the acquisition of mechanistic knowledge.

Another critical issue is related to the nature of bioprocess models. They are in general complex non-linear dynamical systems, involving kinetic parameters that must be identified for the actual cultivation conditions. Due to the complex non-linear structure

of such models, often the parameters involved are not identifiable. Additionally, from the practical point of view, such an identification would require data from specific experiments which are themselves difficult to design and to realize. As such, the costs of parameter identification are in general high.

For these reasons bioprocess mathematical models are often viewed with pessimism in industry. This lack of acceptance of models is strongly reflected on the way process improvements are achieved: these improvements are often achieved by more or less educated trial and error methods guided by intuition and experience, and seldom by a systematic approach characterised by a consequent utilisation of the *a priori* knowledge available. It is well-known that there is a certain inertia on introducing new operation strategies in production plants. Process operators and scientists are looking at the process with different points of view. Operators try to keep the process under control with their experience and intuition, while engineers and scientists try to introduce new and meaningful operation strategies, but are often unaware of the practical implementation problems they involve. This situation is, of course, not optimal.

To increase the acceptance of a model-based way of process improvement it is important to foment a cooperative work between all the factors producing knowledge or insight about the process, i.e. process operators, scientists and engineers, and technology. The models used to describe this knowledge should be capable of incorporating sources of knowledge other then the mechanistic one. Two other sources of knowledge are very important:

1) The heuristic knowledge is very important in the industrial environment since usually process improvement is guided by experience and intuition. In general, heuristic knowledge is available in large quantities in the industrial environment.
2) Knowledge hidden in process data records. Many mechanisms have been explained neither mechanistically nor heuristically. However their cause/effect relationships have been recorded in process data files. Modelling methods like ANNs can be used to extract this kind of knowledge hidden in process data records.

Efficient integration of all the sources of knowledge in the process model is the straightforward way of increasing the benefits and of reducing the expenses. As such the hybrid modelling approach is most promising for industrial applications.

Hybrid modelling is emerging as a new research field. Unfortunately there is no general framework for developing bioprocess hybrid models. One important aim of the present Ph.D. thesis was to develop the hybrid modeling approach, and specially its application for biochemical processes improvement. There are four main issues in hybrid modeling:

1) Hybrid model structure definition
2) Parameter identification in hybrid models
3) On-line adaptation in hybrid model-based algorithms
4) Practical implementation in an industrial environment

The first three topics concern methodologies while the fourth topic concerns mainly software solutions.

Hybrid model structures, parameter identification and on-line adaptation are related topics. In the present Ph.D. work a general framework for hybrid model structure definition, parameter identification and on-line adaptation was proposed based on hybrid model network structures.

There are several reasons that justify the use of hybrid model networks. The first one respects its network structure. The different kinds of knowledge about different parts of the process can be represented and integrated in a most flexible way by means of a network of modules, when these modules are capable of representing knowledge at different levels of sophistication. The network structure is further very flexible for defining special purpose model-based algorithms such as control systems, state estimators and parameter estimators. It is well-known that such a modularization of a process model immediately enhances the transparency of the model and as such helps to avoid erros. Another essential advantage is that such a structure simplifies the practical modeling work by allowing to make use of predefined software modules that need to be adapted only slightly to fit into the model.

The other relevant reason is that hybrid networks, like other network-like structures, belong to the class of ordered systems to which the techniques of forward propagation and error backpropagation can be applied. As such, the learning methods based on error backpropagation, which are most popular for neural networks applications, can also be applied to the case of hybrid model networks. Notice that an artificial neural network is viewed as a particular module in the hybrid model network, in the same way as the sigmoid function is viewed as a particular node in the artificial neural network. Hybrid model networks for biochemical processes are often of considerable complexity. Such models contain many parameters. Consequently, a considerable amount of data is required to identify the model parameters. In such cases, the computing time becomes an issue. The application of optimization algorithms employing gradients estimates obtained by the error backpropagation technique, proved to reduce significantly the computation time for parameter identification of hybrid model networks.

Based on this parallel between hybrid model networks and artificial neural networks, many algorithms usually applied for artificial neural networks were extended and applied to hybrid networks:

1) Error backpropagation
2) Sensitivities method
3) Clustering algorithms
4) Batch/serial learning
5) Cross-validation and regularisation validation strategies
6) Genetic algorithm, chemotaxis and evolutionary programming

These developments allowed to extend and simplify the practical application of hybrid modeling techniques to biochemical process optmization, control and supervision.

The widespread use of the results of advanced methods for process optimization and control is largely delayed by missing software tools that can help to keep the expenditures for development and maintenance in acceptable limits. This problem is

even more critical for the implementation of hybrid model-based methods. In the present Ph.D. work the HYBNET software package was developed to cope with this problem. HYBNET implements all the methodologies based on HYBrid NETworks discussed above. The software was designed to provide all the tools necessary for optimization supervision and control of industrial processes. A very important goal was the development of a nearly platform independent and easy to implement link between hybrid network based algorithms and the process. This, jointly with an user-friendly graphical interface, are recognised to be decisive pre-requisites for a good acceptance in the industrial environment. HYBNET has been used in different biochemical production processes as well as in laboratories and pilot plants. It is continuously being extended in particular concerning the user interface, which is necessary to reduce the activation barrier felt by many process engineers in industry for using software tools.

The other important aim of the present Ph.D. work was to develop methods for rational and efficient use of on-line process information to cope with the impossibility of employing process models describing fully the dynamics of the process. When on-line information is available, it is possible to use a compromising solution, employing algorithms based in simplified models, complemented with on-line adaptation schemes. Such a compromising approach was studied in this work. The development of models assuming no knowledge about the microorganisms growth kinetics is rather simple and, consequently, effort was put on developing strategies for on-line estimation of reaction kinetics from data available on-line. With this respect two stable and easy to tune on-line reaction rates estimation algorithms have been developed. They explore the relationships between stability and dynamics of convergence, imposing convenient second-order trajectories for the estimation errors. Tuning requires only the setting of the parameters characteristic of second-order responses - the damping coefficient and the natural period of oscillation. This represents a lower development cost than the cost of the usual 'trial and error' techniques employed in the daily practice of industrial process operation. However it is worth to notice that the design of special-purpose robust and stable adaptive algorithms is a work that takes very much time and effort, requiring highly qualified manpower. These classical techniques are very powerful and have a well-established theoretical background. However, the real danger persists that their complexity hinders the practical application at reasonable costs to industrial production plants.

The general solution proposed in this Ph.D. thesis to increase the benefit/cost ratio of a model-based process optimization, supervision and control is schematically sketched in Fig 1. This solution is based on a rational and efficient use of all knowledge sources, usually available about the process in question. This implies a cooperative work between all factors producing knowledge, i.e. scientists, process operators, and technology. The more knowledge is becoming available the more accurate are the hybrid models, and the more efficient are the new developed hybrid model-based operating strategies. The rate of process improvement can be significantly augmented by using such an approach.
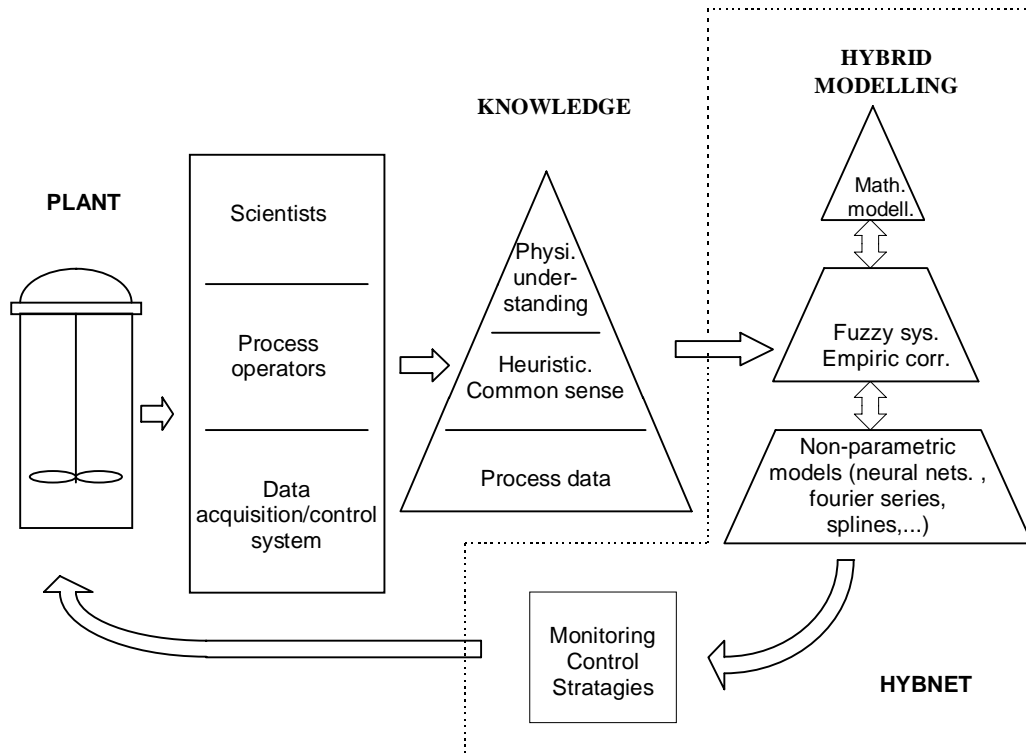
Fig. 1. General approach for biochemical processes supervision, control and optimization based on HYBrid model NETworks and HYBNET software package

This approach has been successfully used in many industrial production plants

1) Closed-loop control in a penicillin production process (Gist Brocades) (http:/rrzn.uni-hannover.de/nhcholiv/bube; Oliveira *et al.*, 1998)
2) Open-loop control in a penicillin production process (Gist Brocades) (Preusting *et al.*, 1997)
3) Process design of a complex antibiotic cultivation process (Gist brocades) (in progress)

As well as in some Lab-scale applications:

4) Open-loop control and state estimation in an ecoli cultivation process for recombinant protein production (in progress)
5) State estimation, optimization and control in a yeast cultivation process (Schubert *et al.*, 1994)

Finally it is important to stress that the most obvious thing to do in order to keep the benefit/cost-ratio as high as possible, is to define very clearly the problem being solved in terms of an process/model objective function (see the work of Simutis *et al.*, 1997). Care should be taken to avoid modeling phenomena irrelevant to the objective function in question. Usually the cost of developing a model is an exponential function of accuracy, hence the desired quality of the model should be also beforehand defined.

REFERENCES

Oliveira, R., R. Simutis, S. Feyo de Azevedo, A. Lübbert (1998). Closed-loop Control Using an On-line Learning Hybrid Model Network (submitted)

Preusting, H., J. Noordover, R. Simutis, A. Lübbert (1997). The use of Hybrid modeling for the optimization of the penicillin fermentation process, *Chimia*, **50(9)**, pp. 416-417

Schubert, J., R. Simutis, M. Dors, I. Havlik, A. Lübbert (1994). Hybrid Modelling of Yeast Production Processes – Combination of a priori knowledge on Different levels of Sophistication. *Chem Eng. Technol.*, **17**, pp. 10-20

Simutis, R., R. Oliveira, M. Manikowski, S. Feyo de Azevedo, A. Lübbert (1997). How to increase the performance of models for process control and optimization. *J. Biotechnol.,* **59**, pp. 73-89

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Publications	15 Publications

Publications list

(15 Publications)

[1] Oliveira, R., E. Ferreira, F. Oliveira, S. Feyo de Azevedo (1994). A Study on the Convergence of Observer-Based Kinetics Estimators in Stirred Tank Bioreactors. 5th International Symposium on Process Systems Engineering - PSE'94, Kyongju, Korea, May 30-June 3

[2] Oliveira, R., E. C. Ferreira, S. Feyo de Azevedo (1995). Model-Based Estimation of Reaction Rates in Stirred Tank Bioreactors. 6th Int. Conference on Computer Applications in Biotechnology - CAB6, Garmisch-Partenkirchen, Deutschland, May 14-17

[3] Oliveira, R., E. Ferreira, F. Oliveira, S. Feyo de Azevedo (1996). A Study on the Convergence of Observer-Based Kinetics Estimators in Stirred Tank Bioreactors. *J. Process Control,* **6(6)**, pp. 367-371

[4] Oliveira, R., R. Simutis, S. Feyo de Azevedo, A. Lübbert (1996). HYBNET, a new tool for advanced process modeling, Proceedings of the 1st European Symposium on Biochemical Engineering Science, Dublin, Ireland, 182-183.

[5] Oliveira, R., N. Volk, R. Simutis, A. Lübbert (1998). Hybride Methoden zur Optimierung biotechnischer Produktionsprozesse. Messen , Steuern, Regeln, (in press)

[6] Oliveira, R., S. Feyo de Azevedo, R. Simutis, A. Lübbert (1998). Hybrid Networks: A New Approach for Bioprocess Modeling (submitted)

[7] Oliveira, R., R. Simutis, A. Lübbert (1998). Closed-loop Control Using an On-line Learning Hybrid Model Network (submitted)

[8] Oliveira, R., S. Feyo de Azevedo (1998). On-Line State Observation and Reaction Rates Estimation in a Baker´s Yeast Cultivation Process. (submitted)

[9] Oliveira, R., R. Simutis, S. Feyo de Azevedo, A. Lübbert (1998). Hybnet, an Advanced Tool for Process Optimization and Control. . 7th Int. Conference on Computer Applications in Biotechnology – CAB7, Osaka, Japan, May 31-June 4, 1998

[10] Feyo de Azevedo, S., R. Oliveira, F. Oliveira (1993). DIGICON - A Generalized Feedback-Feedforward DIGital CONtroller. CHEMPOR-93, Int. Chem Engng Conf., Porto, April 4-6

[11] Ferreira, E., R. Oliveira, F. Oliveira, P. Pimenta, S. Feyo de Azevedo (1993). Model-Based Identification and Control on Baker's Yeast Fed-Batch Fermentation. 6th European Congress on Biotechnology, Firenze, Italy

[12] Simutis, R., R. Oliveira, A. Lübbert (1996). Hybrid modeling with neural networks and its utilization in bioprocess control. In: Bioreactor Engineering. Course notes (Eds)., Larsson, G., Förberg, C., EFB, Saltsjöbaden, Stockholm, pp. 380 - 406.

[13] Simutis, R., R. Oliveira, M. Manikowski, S. Feyo de Azevedo ,A. Lübbert (1997). How to increase the performance of models for process optimization and control. *J. Biotechnol.,* **59**, pp. 73-89

[14] Simutis, R., R. Oliveira, A. Lübbert (1996). Model - based optimization: how to increase the performance of hybrid models for cultivation control, Proceedings of the 1st European Symposium on Biochemical Engineering Science, Dublin, Ireland, 31.

[15] Simutis, R., R. Oliveira, N. Volk, A. Lübbert (1997). Optimierung der Produktion rekombinanter Proteine mit Hilfe Hybrider Prozeßmodelle, Referate der DECHEMA Fachausschußsitzung GVC-Reaktionstechnik, Würzburg, Deutschland, 4.04.