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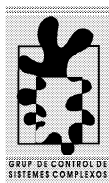
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# Nonlinear robust control of biotechnological processes.

Application to fed-batch bioreactors.

September 21, 2007



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A Aitana. No sigues innocent.  
Fes país. Guanya diners.



## Preface

Engineers usually deal and are identified with electric drives, combustion engines, robots, aircraft and this sort of thing. Biological processes are a completely unknown terrain for an engineer. A smooth and successful landing would have been impossible without the help of Jose Manuel Bruno. But once there, not much would have been done without David, Mercedes, Emma and Chari. And last but not least, I should not miss Carlos out. He is an excellent librarian who luckily decided Brussels is too rainy.

València, 2004



# Abstract

The thesis is centred on fed-batch bioreactors, given the importance of these high density stirred tank reactors for efficient industrial production of proteins, enzymes, . . . using genetically modified microorganisms. The real problem is characterized by the scarcity of on-line measures. Other important problems are the (strong) parametric uncertainty and the presence of significant nonlinearity. Besides the aforementioned problems, in the case of fed-batch bioreactors it is necessary to deal with partial equilibria, i.e. only with respect to some of the variables. The main goals of this thesis are:

1. The search for a limited set of model structures representing most cases of industrial interest.
2. The solution, as a first step in a bottom-up approach, to the control problem for the case of pure cultures with only one limiting substrate and assuming oxygen is in excess.
3. The design of controllers for regulating the microorganisms specific growth rate. Using only biomass and volume on-line measurements with no estimation of the growth rate nor any other variable. And, finally, allowing for the system nonlinearities, uncertainty, and other phenomena.
4. To treat the aforementioned problem of partial stability.

The latter point conditioned the choice of possible techniques to be studied in order to solve the control problem. Namely:

- *Geometric control techniques* (by Fradkov et al.). The problem of bioreactor control can be regarded as a problem of coordinating control. The solution is connected with some specific system properties such as *invariance* and local *attractivity* of nontrivial sets in state space.
- *Flatness*. Although commonly associated to exact linearization, a system can be flat in a subset of the state space with no equilibrium point inside. Even in this case, flatness is still a very useful concept since it allows one to compute a feedforward control algebraically and indicates the system has a very particular structure.
- *Partial stability*. Some results from sources providing Lyapunov-like theorems for partial stability have been used in the stability proofs.

Several important goals have been attained:

1. The determination, after an extensive literature search, of a reduced number of *standard* models for pure cultures with one limiting substrate.
2. An invariant control has been suggested that provides the basis for subsequent designs. Besides, a global stability proof was obtained that enabled the treatment of those problems in which Haldane-like kinetic functions are present.
3. Several designs for the regulation of the specific growth rate are suggested.
4. Finally, several experiments have been carried out using a strain of *S. cerevisiae* with good results.

The attainments in this thesis provide the basis for a future treatment of systems with an inhibitor product, multisubstrate systems, among others.



## Resum

La tesi està centrada en els bioreactors en “fed-batch”, donada la importància d’aquests reactors agitats d’alta densitat per la producció industrial eficient de proteïnes, enzims, ... emprant microorganismes modificats genèticament. El problema real ve caracteritzat per la manca de mesures en línia. Altres problemes importants són la (forta) incertesa paramètrica i la presència significativa de no linealitats. A més dels problemes ja esmentats, en el cas de bioreactors en “fed-batch” és necessari tractar amb equilibris parcials, és a dir, sols respecte a una part de les variables. Els objectius principals de la tesi són:

1. La cerca d’un conjunt limitat d’estructures de models representant la major part dels casos d’interés industrial.
2. La solució, com a primer pas en una aproximació de “baix-a-dalt”, al problema de control per al cas de cultius purs amb un sol substrat limitant i assumint l’oxigen és en excés.
3. El disseny de controladors per regular la taxa específica de creixement dels microorganismes. Emprant sòlament mesures en línia de biomassa i volum, sense cap estimació de la taxa de creixement ni de cap altra variable. I, finalment, tenint en compte les no linealitats del sistema, l’incertesa i altres fenòmens.
4. Tractar el problema esmentat adès d’estabilitat parcial.

El darrer punt ha marcat la tria de possibles tècniques a ser estudiades per tal de resoldre el problema de control. Específicament:

- *Tècniques de control geomètric* (Fradkov et al.). El problema de control de bioreactors pot ser vist com un de “control coordinant”. La solució està relacionada amb algunes propietats específiques dels sistemes com ara *invariança* i *atractivitat* local de conjunts no trivials a l’espai d’estats.
- *Flatness*. Encara que comunment associada amb la linealització exacta, un sistema pot ser pla dins d’un subconjunt de l’espai d’estats sense cap punt d’equilibri a l’interior. Fins i tot en aquest cas, segueix sent un concepte molt útil ja que permet calcular algebraicament una prealimentació i indica que el sistema té una estructura molt particular.

– *Estabilitat parcial*. Alguns resultats, provinents de fonts aportant teoremes tipus Lyapunov per a estabilitat parcial, han sigut emprats en les demostracions d'estabilitat.

S'han assolit diversos resultats importants:

1. La determinació, després d'una cerca extensiva a la literatura, d'un nombre reduït de models estandard per a cultius purs amb un substrat limitant.
2. S'ha suggerit un control invariant que aporta la base per a desenvolupaments posteriors. A més, s'ha obtingut una prova d'estabilitat global que ha permès el tractament de problemes en què apareixen funcions de cinètica tipus Haldane.
3. S'han suggerit diversos dissenys per a la regulació de la taxa específica de creixement.
4. Finalment, s'han portat a terme diversos experiments emprant una variant del *S. cerevisiae* amb bons resultats.

Els assoliments d'aquesta tesi aporten la base per al tractament de sistemes amb producte inhibidor, sistemes multisubstrat, i d'altres.

## Resumen

La tesis está centrada en los biorreactores en “fed-batch”, dada la importancia de estos reactores agitados de alta densidad para la producción industrial eficiente de proteínas, enzimas, ... utilizando microorganismos modificados genéticamente. El problema real está caracterizado por la escasez de medidas en línea. Otros problemas importantes son la (fuerte) incertidumbre paramétrica y la presencia significativa de no linealidades. Además de los problemas comentados, en el caso de biorreactores en “fed-batch” es necesario tratar con equilibrios parciales, es decir, sólo respecto a una parte de las variables. Los objetivos principales de la tesis son:

1. La búsqueda de un conjunto limitado de estructuras de modelos representando la mayor parte de los casos de interés industrial.
2. La solución, como primer paso en una aproximación de “abajo-arriba”, del problema de control para el caso de cultivos puros con un sólo substrato limitante y asumiendo el oxígeno está en exceso.
3. El diseño de controladores para regular la tasa específica de crecimiento de los microorganismos. Utilizando sólo medidas en línea de biomasa y volumen, sin ninguna estimación de la tasa de crecimiento ni de ninguna otra variable. Y, finalmente, teniendo en cuenta las no linealidades del sistema, la incertidumbre y otros fenómenos.
4. Tratar el problema citado anteriormente de estabilidad parcial.

El último punto ha marcado la elección de las posibles técnicas a estudiar para resolver el problema de control. En concreto:

- *Técnicas de control geométrico*(Fradkov et al.). El problema de control de biorreactores puede ser visto como uno de “control de coordinación”. La solución está relacionada con algunas propiedades específicas de los sistemas como la *invarianza* y la *atractividad* local de conjuntos no triviales en el espacio de estados.
- *Flatness*. Aunque comunmente asociada con la linealización exacta, un sistema puede ser plano dentro de un subconjunto del espacio de estados sin ningún punto de equilibrio en su interior. Incluso en este caso, sigue siendo un concepto muy útil ya que permite calcular algebraicamente una prealimentación e indica que el sistema tiene una estructura muy particular.

– *Estabilidad parcial*. Algunos resultados, provenientes de fuentes aportando teoremas tipo Lyapunov para estabilidad parcial, han sido utilizados en las demostraciones de estabilidad.

Se han conseguido varios resultados importantes:

1. La determinación, después de una búsqueda extensiva en la literatura, de un número reducido de modelos estandard para cultivos puros con un sustrato limitante.
2. Se ha sugerido un control invariante que aporta la base para desarrollos posteriores. Además, se ha obtenido una prueba de estabilidad global que ha permitido el tratamiento de problemas en los que aparecen funciones de cinética tipo Haldane.
3. Se han sugerido diversos diseños para la regulación de la tasa específica de crecimiento.
4. Finalmente, se han realizado varios experimentos utilizando una cepa del *S. cerevisiae* con buenos resultados.

Los resultados de esta tesis aportan la base para el tratamiento de sistemas con producto inhibitor, sistemas multisustrato, y otros.

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# 1 General Introduction

The Group of Complex Systems Control, now a CSIC-associated unit, has been collaborating with the Pilot Plant of Biotechnology<sup>1</sup> in the IATA (CSIC)<sup>2</sup> for a long time. Their main interest is in fermentation processes for the production of proteins, enzymes . . . using genetically modified microorganisms or *recombinants*. This kind of application represents a significant proportion of all industrial uses of biotechnology. Some of them, such as the production of antibiotics and other pharmaceutical products, are more and more important.

All these processes are carried out using several kinds of reactors which can be classified under two main groups: stirred tanks and tubular or column ones. The latter, not dealt with here, must be described taking into account diffusion phenomena and consequently using PDEs. In the former, homogeneous conditions can be assumed at least for lab-scale or small-scale tanks, thus allowing for description using ODEs. Further to this classification, reactors can also be divided into low and high-density ones. The latter being clearly preferred for production. The most important of them is the fed-batch bioreactor, which is also of the stirred tank class. Hence, all the work is centred on this particular kind of bioreactor.

Most theses are set to solve a given theoretical problem within a given theoretical framework. Afterwards, applications are sought after to illustrate the main subject. In the work reported, a different procedure has been followed. From the very beginning, solving a real problem was the main goal. Particularly, the design of controllers, for improving the productivity in the aforementioned fed-batch bioreactors. No theory or technique was chosen *a priori*. It was expected this choice would present itself as research with the bioreactors proceeded.

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<sup>1</sup> Now Biopolis S.L. ([www.biopolis.es](http://www.biopolis.es)), a company shared by the CSIC, Natraceutical, Central Lechera Asturiana and Talde.

<sup>2</sup> Inst. of Agrochem. and Food Tech. - Spanish Council for Scientific Research

## Objectives

The main objective can be subdivided into the following:

1. The search for a limited set of model structures representing most cases of industrial interest. Thus, simplifying the search for solutions while ensuring their usefulness.
2. The solution, as a first step in a bottom-up approach, to the control problem described below for the case of pure cultures with only one limiting substrate and assuming oxygen is in excess.
3. All applicable regulators must use as few measures as possible. In our case only biomass and volume measurements with no estimation of the growth rate nor any other variable. Thus facilitating the practical implementation of the regulators, given that the real problem is characterized by the scarcity of on-line measures. A key state variable, the substrate (nutrient) concentration in the broth, is as a rule difficult to measure on-line. Obviously, another line of research may have tried to design robust observers for non-linear systems. In practice, these observers may not be adequate. For example, in many cases the substrate concentration is so low that it is within the order of magnitude of noise. Finally, it is also interesting in itself to analyze what can be done with a minimum of information.
4. The controllers must also be quite robust since the presence of (strong) parametric uncertainty and significant nonlinearity are common problems in this kind of process.
5. The main control specification: regulating the microorganisms specific growth rate. A goal usually associated by the biologists with the maintenance of a definite physiological state. It also appears in applications of optimal control to biotechnological processes.
6. Besides the previously mentioned problems, in the case of fed-batch bioreactors, partial equilibria and unbounded reference signals must be dealt with. Specifically, a constant growth rate implies an exponential trajectory for the absolute quantity of biomass.

## Structure

The solutions suggested in this thesis are presented and explained in the subsequent chapters, which are structured as follows:

- *Problem statement - Ch 2.* As shown in chapter 2, there are two standard models or structures that represent at least 95% of all pure cultures of industrial interest. There are articles using these structures even for processes with *mixed cultures*. The case of multi-substrate pure cultures is also dealt with in this chapter, but apart from this it has been left aside. There are already some studies on the modelling and control of general biotechnological



processes that, besides the aforementioned ones, also include different kinds of recycling plants (e.g: wastewater treatment), etcetera. In a sense, these models are too general for our purposes. The biologists suggested there is a limited number of *typical* microorganisms showing different kinds of behaviour. It must be taken into account that most often the microorganisms to be genetically modified are chosen for their simple and/or well-known behavioural patterns. Hence, a reduced set of models may be used and their particularities exploited for control purposes. An extensive and intensive literature search confirmed this point. Many aspects of the control problem as a whole, such as the presence of nonlinearity and uncertainty, are also presented in this chapter.

- *Invariant control - Ch 3* and *Dealing with uncertainty - Ch 4*. The search for new techniques able to cope naturally with problems involving partial equilibria led to the study of different theories. Namely *flatness* and techniques considering *partial stability*. The former is studied in Appendix A and its applications for control shown in chapters 3 and 4. Although commonly associated to exact linearization, these are different properties which under certain circumstances coincide. In fact, a system can be flat in a subset of the state space with no equilibrium point inside. Even in this case, flatness is still a very useful concept since it allows one to compute algebraically a feedforward control and indicates the system has a very particular structure. The other techniques considered consist of the geometric control techniques suggested by Fradkov et al. in [134] and their applications are also considered in the aforementioned chapters. Besides the reasons exposed above, other factors make this geometric theory quite suitable. In particular, the control specifications translate into a problem of regulating a function (the *specific growth rate*) of part of the state variables. Consequently the problem of bioreactor control can be regarded as a problem of coordinating control where coordination conditions, given in the form of a relation of output variables, define smooth surfaces or multidimensional submanifolds of the output space. These have a translation into state space. The non-trivial geometrical objects obtained in this way are usually called *goal sets (submanifolds)*. The solution of the control problem is then connected with some specific system properties such as *invariance* and local *attractivity* of nontrivial sets. The controller derived using flatness has a similar structure, one invariant control plus a stabilizing law. Thus, a common structure was found which suggested the division between chapter 3 *Invariant control* and chapter 4 *Dealing with uncertainty*. Building on the ideas developed in these stages, a new nonlinear robust adaptive controller was designed. See again chapter 4.
- *Practical results - Ch 5*. Finally, chapter 5 presents some experimental results on real fermenters.
- *Conclusions - Ch 6* and the *Appendices*, including a survey on flatness and some additional documentation.

### Attainments

Several important goals have been attained:

1. The determination of a reduced number of *standard* models of bioreactors after an extensive literature search. These models have relatively few state variables and a couple of particular structures represent most applications for pure cultures with one limiting substrate.
2. An invariant control has been suggested that provides the basis for subsequent designs. In particular, it is a part of the controllers presented in chapter 4. Besides, a global stability proof was obtained that enabled the treatment of those problems in which Haldane-like kinetic functions are present.
3. Three designs for the regulation of the specific growth rate are suggested. The first one is based on flatness and uses two PIs. The second one is based on the geometric control techniques developed by Fradkov et al. It has been shown that the problem can be cast as one of partial stability, and the corresponding techniques have been used to analyze it. Using many elements and ideas of this design, a robust adaptive controller has been developed and its stability proved.
4. Finally, several experiments have been carried out using a strain of *S. cerevisiae* with good results.

The attainments in this thesis provide the basis for a future treatment of systems with an inhibitor product, multisubstrate systems, among others.

### Final remark

Before proceeding with chapter 2, a remark on nomenclature is in order. Strictly speaking the term *fermentation* should be used with caution, but in practice it is used (or misused) for almost any kind of bioreaction. This usage is reflected in the text.

## 2 Problem statement

Biological systems are an example of complex systems. When being considered as a control plant, complex dynamical systems are characterized by [134]:

- High state-space dimension
- Multiple inputs and outputs
- External disturbances ...
- Significant nonlinearity and uncertainty
- Sophisticated and multiple objectives and performance criteria.

All these traits can be found in the bioreactors and are dealt with in the following sections. This chapter is focused on an in-depth study of bioreactor modelling for control purposes, considering not only the aspects mentioned above but also the different production modes, available control inputs and measurable outputs. In the literature, many different models for biotechnological processes can be found. They vary not only in the functions used to model the reaction kinetics but also in the structure and kind of the model equations. For control purposes, the so called *unstructured* and *non-segregated* models are mostly chosen. Even restricting ourselves to this kind of models, there is still an important variety. Some authors, e.g. [1], suggest very general models accounting for many different situations. In the approach adopted in this chapter, a limited number of simple models considering as few variables as possible is sought after. A set of *standard models* for the so called pure cultures, already suggested by other authors, is presented. Their representativity is judged on the basis of an extensive review of the models appearing in the literature and an analysis of a series of basic concepts, namely biomass, substrate and product. As an introduction, the classical approach to the subject is briefly developed. Afterwards it is shown in a reasoned way that the above mentioned standard models actually represent most processes of practical interest. The chapter is complemented by one section dedicated to kinetic functions. Finally, the reader will find a *catalogue* with real examples and references, besides some comments on structured/segregated models.

## 2.1 Introduction

A complete model of a bioreactor may have to address mass transfer, growth and biochemistry, physical chemical equilibria and various combinations of each of these. It becomes hard to write simple equations when an accumulation of factors affect time behaviour, but it is possible to develop differential equations with terms for important factors. In other words, reduce a system to its main components and formulate mass balances and rate equations that integrate overall behaviour. This point is developed in the present section.

A bioreactor can be defined as *a tank in which several microbial growth<sup>1</sup> and enzyme-catalyzed reactions<sup>2</sup> occur simultaneously in a liquid medium.*

There are different kinds of models [2], depending on the simplifications considered:

- If the cell is regarded as a *black box* and only the main extracellular species consumed or excreted in the medium are considered, without delving into the intracellular mechanisms, then the model is said to be *non-structured*, otherwise structured.
- Cells may be subject to several phenomena such as *aging* so not all of them have the same capacity of division, or genetic mutation so that some cells do not produce the species of interest (plasmid loss). But if the model is built supposing an average cell then it is said to be *non-segregated*.

Most fermentations of interest can be properly described, at least for control purposes, by means of unstructured and non-segregated models. The possible uses of structured and/or segregated models are commented in the final section. Another simplification often considered, as previously mentioned, is:

- *Homogeneity*. The conditions and concentrations in the tank are supposed to be homogeneous, which is a good approximation for lab-scale and pre-industrial fermentors.

From these hypotheses we proceed to develop models complete enough to account for the process behaviour but not so complex that they become extremely difficult to handle.

The traditional presentation, described in [1] and also [146], goes as follows:

The growth of microorganisms (bacteria, yeasts, etc.) proceeds by consumption of appropriate nutrients or *substrates* (involving carbon, nitrogen, oxygen, etc.) provided the environmental conditions (temperature, pH, etc) are favourable. The mass of living microorganisms or living cells is called the *biomass*. Associated with cell growth, but often proceeding at a different rate, are the enzyme catalysed reactions in which some reactants are transformed

<sup>1</sup> Often referred to as microbiological reactions

<sup>2</sup> Also termed biochemical reactions or biotransformations

into *products* (sometimes called metabolites) through the catalytic action of intracellular or extracellular enzymes. As an example, take the dynamical behaviour of the growth of one population of microorganisms on a single *limiting substrate*<sup>3</sup> in a stirred tank reactor. It is most often expressed by the following equations which are obtained from straightforward mass balances.

- The net accumulation of biomass in the reactor

$$\frac{d(vx)}{dt} = \mu vx - F_{out}x \quad (2.1)$$

- The net accumulation of substrate in the reactor

$$\frac{d(vs)}{dt} = -k_1\mu vx + F_{in}s_{in} - F_{out}s \quad (2.2)$$

- The variation of volume

$$\frac{dv}{dt} = F_{in} - F_{out} \quad (2.3)$$

With  $x$  being the concentration of the microbial population (biomass) in the reactor and in the effluent,  $s$  the substrate concentration in the reactor and in the effluent,  $s_{in}$  the substrate concentration in the influent,  $F_{in}$  the influent flow rate,  $F_{out}$  the effluent flow rate,  $\mu$  the specific microbial growth rate,  $k_1$  the yield coefficient of substrate consumption by the biomass, and  $v$  the volume of the culture medium. Some models may also consider other factors such as gas exchange:

1. The growth of microorganisms in bioreactors is often accompanied by the formation of products which are either soluble in the culture or which are given off in gaseous form. The mass balance relative to the product in the bioreactor is given by:

$$\frac{dp}{dt} = \pi x - Dp - Q \quad (2.4)$$

with  $p$  being the synthesis product concentration (in the liquid phase),  $Q$  the rate of mass outflow of the product from the reactor in gaseous form,  $D = F/v$  the dilution<sup>4</sup> and  $\pi$  the specific production rate.

2. Dissolved oxygen dynamics in aerobic fermentations, i.e. those in which microorganisms need oxygen to develop properly. The dissolved oxygen (DO) mass balance in the bioreactor is described as follows:

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<sup>3</sup> For the time being, suffice it to say this is in a way the main substrate or that one directly influencing the growth rate.

<sup>4</sup> Here, it is considered that  $F = F_{in} = F_{out}$  or  $F = F_{in} \quad F_{out} = 0$

$$\frac{dC}{dt} = OTR - OUR - DC \quad (2.5)$$

where  $C$  is the DO concentration in the reactor,  $OTR$  is the oxygen transfer rate and  $OUR$  is the oxygen uptake rate. Expressions for these terms can be found in [1].

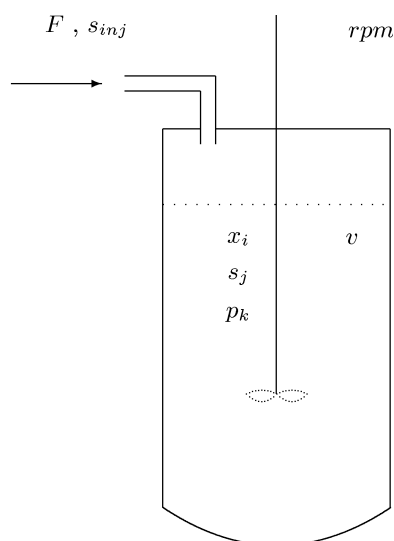
The way  $F_{in}$  and  $F_{out}$  are used depends on the chosen production mode. On the basis of liquid medium one-stage bioreactors the following modes are found:

1. *Batch*. The simplest one. There is no material exchange with the environment except for gasses (oxygen, carbon dioxide,..), i.e.  $F_{in} = F_{out} = 0$ . All substrates are in excess within the reactor from the beginning of fermentation.
2. *Chemostats* and *auxostats*, also termed *continuous bioreactors*. The reactor is continuously fed with a substrate influent. There is also an outflow whose rate is equal to the inflow rate ( $F_{in} = F_{out} = F$ ), hence the volume is constant for a fixed dilution. In the chemostat, the nutrient is fed at a constant rate, i.e. the dilution is  $D = F/v = const$ , which implies in steady state a constant cell division rate and thus a constant physiological state. This device was invented in the early 1940's and marked the advent of serious continuous fermentation [143].

An auxostat, a name coined by Martin and Hempfling in 1976, also called nustat or nutritstat, can be regarded as a chemostat plus a control feedback. Medium with a given substrate concentration is fed in a suitable way into the reactor so as to hold constant a given factor such as the pH, dissolved oxygen or a particular concentration. From a biologist's point of view, it could be said that "the microorganisms establish the feeding rate as it is adjusted to match their rate of metabolism". Particular cases reported in the bioprocess and bioengineering literature are the pH-auxostat, the DO-auxostat and the turbidostat. Clearly, the same ideas can be used in fed-batch bioreactors.

Continuous reactors have two important disadvantages. First, the low efficiency with respect to substrate usage. Second, a higher risk of contamination. Hence, continuous reactors are less used in industry, with the exception of waste treatment processes. Researchers often use them to determine certain physiological parameters of microorganisms.

3. *Fed-batch*. Usually the production is carried out in fedbatch mode since substrate usage is optimized. The basic scheme is shown in figure 2.1.



**Fig. 2.1.** Fed-batch bioreactor

The symbols in figure 2.1 read as follows:

- There can be several substrates  $s_j$  (multi-substrate systems), products  $p_k$  and possibly different biomass populations  $x_i$  (i.e. mixed cultures). The  $s_j$ ,  $p_k$  and  $x_i$  represent concentrations of the corresponding species in the tank.
- $v$  is the volume in the bioreactor,  $F = F_{in}$  the input flux or alternatively  $D = F/v$  the dilution.
- $s_{inj}$  the concentration of substrate  $j$  in the input flux.
- There is also, in aerobic fermentations, gas exchange with the environment (e.g:  $O_2, CO_2$ ), but often it is not considered unless oxygen is limited.

In general it is difficult to measure the substrate/s  $s_j$  on line. Often the available measures are off-line. On the other hand, there are sensors for biomass<sup>5</sup> even though in general it is not possible to differentiate between different populations within the same reactor. The same applies to the distinction between viable and non-viable cells. Clearly, it is also possible to measure volume  $v$ . Other state variables such as products are seldom available on-line. Use of observers is not trivial either, and estimations of the specific growth rate  $\mu$  tend to be too noisy. A comprehensive survey of available measurements for bioreactors can be found in [142].

As for the specifications, it is usually desirable to make biomass grow at a fixed rate until there is no more substrate to feed or the maximum tank

<sup>5</sup> The group designed a biomass sensor with a good range. See [137].

capacity has been reached. The goal is to keep the microorganism in a given physiological state. As it will be explained later, the specific growth rate depends on several factors and consequently we will have a series of sub-specifications:

- First of all, a set of environmental conditions such as temperature, pH, pO<sub>2</sub>... must be considered. These are usually kept constant at some optimal values.
- Secondly, growth depends on the substrate concentration  $s$  and may also depend on that of a given product  $p$ . In the simple yet frequent case in which it only depends on  $s$ , we have  $\mu = const$  implies  $s = const$ . But substrate concentration is seldom if ever measured on-line. On the other hand, it can be seen that a constant  $\mu$  corresponds with an exponential trajectory for the absolute quantity of biomass  $\bar{x} = xv$  and viceversa. It is interesting to see that only part of the variables reach an equilibrium point (e.g. substrate concentration). Other variables, i.e. volume, follow an unbounded trajectory. This kind of setting is considered within the framework of partial stability analysis. References for this subject are [138] and particularly [92]. In [1] the point is highlighted but it is not dealt with.

This point will be considered again once a set of basis models has been established.

For the time being, equations 2.1 to 2.5 form the basis for a general dynamical model of bioreactors [1]:

$$\dot{\xi} = K\varphi(\xi, t) - D\xi - Q(\xi) + F \quad (2.6)$$

In this model several coexisting microbial populations, substrates, and products can be considered. The different state variables are represented by vector  $\xi$ <sup>6</sup>. The first term  $K\varphi(\xi, t)$  describes the kinetics of the biochemical and microbiological reactions which are involved in the process. The remaining terms describe the transport dynamics of the components through the bioreactor. Functions  $\varphi$  are the rate equations which may depend on several state and environmental variables. These are bounded functions which may be monotonously increasing-decreasing or non-monotonous<sup>7</sup>.

A priori, from this standpoint, we may get models of arbitrary order and still very complex and difficult to handle. But the experience of biologists and biotechnologists shows that in practice there is a reduced number of typical systems. This motivated a review of the question. This revision, in turn, entailed a deeper study of the three key concepts: biomass, substrate and product. This study is presented in the following section.

<sup>6</sup> Notice  $\dot{\xi}$  stands for  $d\xi/dt$ . From now on, this will be used as standard notation

<sup>7</sup> See section *Kinetic functions* for further details



## 2.2 Basic concepts

This section is divided into three blocks dedicated to the basic concepts mentioned above:

### Biomass

Defined as the concentration of living cells in the bioreactor, although it may refer also to the population of microorganisms as a whole. Usually, populations formed only by one species or strain are dealt with. These are the so-called *pure cultures*. In some instances, there may be more than one species. These are the so-called *mixed cultures*. Under this heading several possible interactions between two or more microorganisms are considered [146]. References in the literature to processes using several microorganisms for the production of enzymes, proteins, antibiotics...are not common. Mixed cultures of industrial interest are:

- a) A particular case of mixed culture in which there is a population of genetically modified microorganisms and a second one formed by microorganisms which have lost the modification. This phenomenon is referred to as plasmid loss [74].
- b) Waste treatment processes, e.g. waste-water treatment processes. In this case there are many organisms living on many different and varying substrates. Being a field in its own right, it has not been considered in this work.

Case (a) is more interesting for our purposes and falls under the general case of several microorganisms competing for a single nutrient source. Recent studies [74] show that even if we interfere in the system it is not possible to have more than two different populations coexisting in equilibrium.

Other possible relations between two microorganisms, such as predator-prey kinetics or commensalism, are listed in [146].

### Substrate

The case for only one *main* substrate is presented first. The multisubstrate case is much more complex, encompasses several situations and it is in fact hard to find in the literature a unified view on the subject. Thus, two presentations are given along with their coincidences. Illustrative examples are shown in section 2.5.

Microorganisms actually live on a combination of carbon sources, nitrogen sources, vitamins and other elements which altogether form the culture medium. These different substrates may be divided into:

- **homologous**: substrates serving the same physiological purpose, e.g. the substrates considered are all carbon sources or nitrogen sources.

- **heterologous:** substrates serving different physiological purposes. In this case each substrate satisfies a different metabolic requirement, e.g. one substrate serves as carbon source and another substrate serves as nitrogen source.

Typically, all these substrates except one are found in excess both in the medium and the inflow. The nutrient in short supply relative to the others will be exhausted first and will thus *limit cellular growth*. At this point, it should be mentioned that in microbiology the term *limitation of growth* is used in two different ways [148]:

- In a stoichiometric sense, i.e. it indicates that a certain amount of biomass is synthesized from a particular nutrient. This is reflected in the growth yield constants for the different elements.
- In the sense that the specific growth rate  $\mu$  is controlled by the extracellular concentration  $s$  of a particular growth substrate.

The present discussion is limited to the second case. The ingredients, other than the limiting one, may play various roles such as exhibiting toxicity or promoting cellular activities, but there will not be an acute shortage to restrict growth as in the case of the limiting nutrient becoming exhausted. Only this *limiting substrate* will take part in the equations, the other ones being disregarded. Which of the previously mentioned elements plays the role of limiting substrate? According to [143], the usual concepts about growth limitation are based mainly on a carbonaceous nutrient such as glucose. The situation changes slightly for other factors such as nitrogen, sulfur and phosphate. The problem arises because cell mass may not exactly reflect these limitations. For example, cells without adequate nitrogenous components will store carbonaceous ingredients for later use....or the ratio of the limiting nutrient to other media components may be critical in the production of many bio-products such as storage compounds, exo-polysaccharides and enzymes.

When dealing with several limiting substrates, several approaches can be taken as mentioned above:

- In [143] a division is made between diauxic and biphasic behaviour. This division seems to make sense only when dealing with two (or more) substrates which are carbon sources. The basic definitions are as follows
  - \* **diauxic:** diauxic consumption of two or more substrates means the substrates are used in sequence. The utilization of the second substrate begins after the first one has been exhausted.
  - \* **biphasic:** multi-substrate limited growth, where more than one nutrient affects the growth rate and are consumed simultaneously.
- In [145] a different approach is taken. Substrates are divided into:
  - \* **essential:** there is no growth if only one of the substrates is lacking. Two cases are comprised here
    - *Interactive model.* All substrates together determine the growth rate.

- *Non-interactive model.* At any time only the substrate with the strongest limitation governs the growth rate. This leads to a selection of the minimum growth rate allowed among all substrates.

A typical example for the presence of two essential substrates is growth of obligate aerobic microorganisms under limitation of the carbon source and oxygen.

- \* **growth enhancing and alternative:** one of the substrates is already sufficient for growth and others are
  - either used up in parallel,
  - or sequentially.

A comparison of both approaches could be summed up in the following scheme:

- The concept of diauxia coincides with that of alternative substrates, i.e. those used up sequentially.
- The concept of biphasia would coincide both with the case of essential substrates in interactive models and the case of growth enhancing substrates, i.e. those used up in parallel.
- Finally, the case of essential substrates in non-interactive models is reducible to a set of models with a single limiting substrate each one.

Some examples of the different behaviours are shown in the catalogue, but some comments are in order here. Many microorganisms show a *diauxic* behaviour, the passage from one substrate to another requiring an adaptation period. Biphasic behaviour is reported in the *S. cerevisiae* [44] and *E. coli* [77], [149]. Besides the case of obligate aerobic microorganisms mentioned above. Finally, an example [37] was found which doesn't seem to fit into any of the classifications presented in this section. In this example, there is only one limiting substrate affecting growth but there is also an *inducer* being used up. The inducer only affects production of a product  $p$ , but this one in turn inhibits growth. Hence it is necessary to consider both species in the model.

## Product

All species excreted by the microorganism may be labelled as products. A simple functional classification may go as follows:

- Products of interest (enzymes, proteins, antibiotics...)
- Inhibitory products, i.e. affecting growth directly. Usually, only one if present and it may coincide with a product of interest.
- A product may be used also as a substrate as mentioned before. Some inhibitory products such as ethanol may take part in these phenomena.

Usually, products are considered in the model only if inhibitory. Although that depends also on the particular case and our purposes. For example, in fed-batch mode and for a given maximum volume several optimization

problems can be set in which the product  $p$  must be known. In some cases of diauxic behaviour, in which a given species is first product and in a second stage substrate, it is easier to have two separate models. The main exception is found in the case of biphasic behaviour.

There is also a classification of products depending on how they are generated by the microorganism. See below and also reference [145].

### 2.3 Models for control

From previous sections it is possible to deduce that as a rule, for control purposes, it is only necessary to consider in the models the following:

1. At most two different populations of microorganisms.
2. No more than two limiting substrates, particularly if oxygen is in excess.
3. One product.
4. And, in some kinds of bioreactors, volume.

A worst typical case may imply:  $x_1, x_2, s_1, s_2, p, v$ . In all six state variables. Additionally, a bibliographic review shows that actually two *standard models* with three or four state variables  $(x,s,p,v)$  cover a huge portion of all applications. These models, corresponding to pure cultures, will be treated carefully below.

The *standard models* presented in this section are unstructured non-segregated models that represent pure cultures with one limiting substrate. In this models gas exchange is not considered and oxygen is assumed to be in excess unless it is the limiting substrate as in example 2.21 in the catalogue<sup>8</sup>. It is also standard practice to consider only one product. Either the metabolite of interest or, if it exists, an *inhibitor*, a product that somehow affects microbial growth. In both cases the product may be considered, for example when applying optimization techniques for productivity enhancement. But, for our purposes, only in the second case the product must always be taken into account.

A high percentage of the bioreactions can be classified into two general types according to the reaction schemes [1], [5]. In the first type, the product is formed in parallel with the microbial growth. It is said to be growth-linked.



The symbol  $\rightleftharpoons$  indicates that the biomass is an autocatalyst, i.e. a catalyst of its own production. The more biomass there is, the more biomass (and

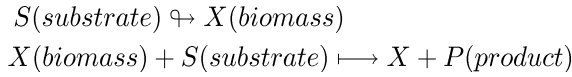
<sup>8</sup> Oxygen often appears in multi-substrate models as a limiting substrate along with a carbon source (e.g. glucose). This sort of models, although briefly reviewed here, are not dealt with in the following chapters

product) can be produced. It is a pseudoreactant which is not consumed by the reaction but can be accumulated in the reactor. The following state space model can be derived:

$$\begin{aligned}
 \dot{x} &= \mu x - Dx \\
 \dot{s} &= -y_s \mu x + D(s_i - s) \\
 \dot{p} &= y_p \mu x - Dp \\
 \dot{v} &= F
 \end{aligned} \tag{2.8}$$

where  $x$ ,  $s$  and  $p$  are the biomass, substrate and product concentrations respectively;  $D$  is the dilution rate;  $s_i$  is the influent substrate concentration;  $\mu$  is the specific growth rate;  $y_s$  and  $y_p$  are the yield coefficients. Processes for the production of single-cell protein, alcohol and gluconic acid all belong to this category.

In the second type processes, product formation takes place either in the final phase of growth or in the secondary way, which is not directly connected with growth. The reaction scheme can be expressed as follows:



from which the following state space model can be derived:

$$\begin{aligned}
 \dot{x} &= \mu x - Dx \\
 \dot{s} &= -y_{sx} \mu x - y_{sp} \pi x + D(s_i - s) \\
 \dot{p} &= \pi x - Dp \\
 \dot{v} &= F
 \end{aligned} \tag{2.9}$$

where  $\pi$  is the specific production rate. Many antibiotics (streptomycin, penicillin), lactic acid, citric acid, itaconic acid, glucoamylase and some amino acids are produced by this type of fermentation.

These two models are commonly taken in the literature as the standard ones for representing fermentation processes. See [4] to [15].

Depending on metabolite production, composition of culture medium and regulation in the strain used, there may be intermediate forms. A more general model [4] may be:

$$\begin{aligned}
 \dot{x} &= \mu x - Dx \\
 \dot{s} &= -\sigma x + D(s_i - s) \\
 \dot{p} &= \pi x - kp - Dp \\
 \dot{v} &= F
 \end{aligned} \tag{2.10}$$

where  $k$  is the hydrolysis (or degradation) constant for product;  $\mu$ ,  $\sigma$  and  $\pi$  are the specific rates of growth, substrate consumption and product formation, respectively. In addition, there may be terms for biomass decay and maintenance substrate consumption in the form  $k_{d/m}x$ , but usually are not taken into account. The specific rates may depend on substrate, cell, and product concentrations, or they may be related to each other. The model (2.10) represents various fed-batch fermentations such as

- microbial cell productions involving bacteria. No metabolite production, both  $\mu$  and  $\sigma$  only depend on  $s$ .
- lysine production.  $k = 0$ ,  $\mu(s)$ ,  $\pi(\mu)$  and  $\sigma(\mu)$ .
- alcohol production.  $k = 0$ ,  $\mu(s, p)$ ,  $\pi(s, p)$  and  $\sigma(s, p)$ .
- antibiotic production.  $k = 0$ ,  $\mu(x, s)$ ,  $\pi(s)$  and  $\sigma(s)$ .

Note that in this list  $\mu$  and the other specific rates depend always on  $s$  and may be on  $x$ , but not on the product except for the case of alcohol production. Consequently, it may happen that production of say an antibiotic which in principle follows model 2.9 may actually be modelled for control purposes by a simpler set of equations discarding the equation for product.

As for the control specifications, two cases may be found depending on whether product affects the kinetic functions or not. Having  $\mu = \mu_r = \text{const}$ <sup>9</sup> will be in the first case equivalent to  $s = s_r$ , whereas in the second case several possibilities are open. A reasonable specification would be to keep a constant product concentration at some  $p = p_r$  along with  $s = s_r$ . Additional devices would be needed for filtering the given product out of the broth.

At this point, it is interesting to make some comments on the control inputs which have been assumed to be available in the control designs presented in later chapters. First of all, the quantity of substrate supplied to the bioreactor depends on two parameters:

- $F$ , the input flux.
- $s_i$ , the substrate concentration in the input flux.

Usually only the first possibility is readily available. Two cases are shown:

1. Only one control action,  $F$  or  $s_i$  is used, resulting in an affine system. If the input flux is changed, a double effect occurs:
  - the dilution  $D$  will change and consequently the concentrations of all the species present in the bioreactor.
  - the quantity of substrate supplied will change also and then, indirectly, the specific growth rate.
2. If an actuator able to vary separately the flux  $F$  and  $s_i$  is available, then it will be possible to take into account independently the two effects

<sup>9</sup> Taking the equation for biomass in absolute masses instead of concentrations,  $\dot{\bar{x}} = \mu\bar{x}$ , it is easily checked that in any case  $\mu = \mu_r$  is equivalent to forcing an exponential trajectory for  $\bar{x} = xv$  as explained in a previous section

enumerated above. In any case, the coupling between the new control actions should be considered. Although a non-affine system is obtained, in practice it is implemented using two input flows  $F_1$  and  $F_2$  with substrate concentrations  $s_{inmax}$  and  $s_{inmin}$  resulting in an affine control system

Finally, if the microorganism produces an inhibitor it is very interesting to add a recirculation through a filter in order to keep the inhibitor concentration at a given value. In a first approach this may be represented in the equations as a new control action  $\alpha$  which determines the rate of product removal. This combination also gives an affine system:

$$\begin{aligned}\dot{x} &= \mu x - Dx \\ \dot{s} &= -\sigma x + D(s_i - s) \\ \dot{p} &= \pi x - \alpha p - Dp \\ \dot{v} &= F\end{aligned}\tag{2.11}$$

This will be used in chapter 3. Of course, for real application further study of the dynamics introduced by the filtering system and its effect on the system should be carried out. In [155] an interesting application of a scheme using medium recycling is presented showing good productivity results.

## 2.4 Kinetic functions

As it has been mentioned previously, the functions  $\mu$ ,  $\sigma$  and  $\pi$  depend on several factors such as the concentrations of substrate and product, but also the pH, temperature, etc.

Usually,  $\mu$  is expressed as a product of several terms and each one depends on one of the factors previously cited. Thus:

$$\mu = \mu(s)\mu(p)\mu(pH)\mu(T)\dots\tag{2.12}$$

Temperature, pH and other environmental variables are usually kept constant. In some cases the production process is divided into several stages or phases, and these variables may have different constant values in each stage. It may also be possible to let a particular variable (e.g: pH, pO<sub>2</sub>) evolve freely in order to, for example, facilitate the adaptation of the microorganisms from one phase to the next. As for the other factors mentioned above the most common expressions are:

– Substrate concentration. (See fig. 2.2)

1. Monod

$$\mu(s) = \frac{\mu_m s}{k_s + s}\tag{2.13}$$

where  $\mu_m$  is the maximum growth rate, and  $k_s$  a transport constant.

2. Haldane, in which inhibition of growth by the substrate is considered.

$$\mu(s) = \frac{\mu_o s}{k_s + s + \frac{s^2}{k_i}} \quad (2.14)$$

A relatively common alternative expression is

$$\mu(s) = \frac{\mu_m s}{k_s + s} \frac{1}{k + s} \quad (2.15)$$

i.e. the product of a Monod-like term and another term representing the inhibition.

In practice there is always an inhibition of biomass growth, but in many cases it appears for substrate concentrations that are very high when compared to those in the zone of interest. Hence, most often a Monod growth rate model is used. Applications such as water decontamination fall into the opposite case. In the fermentation processes we work with, inhibitory effects caused by a product are, if present, more important.

- Product concentration. An example may be the inhibition of growth induced by ethanol.

$$\mu(p) = \frac{k_p}{k_p + p} \quad (2.16)$$

- Biomass concentration. Microbial growth may be slower at high concentrations. A modification of Monod's model, in order to introduce this effect, is the Contois model

$$\mu(s, x) = \frac{\mu_m s}{k_c x + s} \quad (2.17)$$

Another common model is the logistic one

$$\mu(x) = \mu_m (1 - ax) \quad (2.18)$$

There are many others in the literature. For a comprehensive list see [1] and also [145]. In the last reference, it is noted that the differences among the different kinetic functions are less relevant if one keeps in mind measuring errors and remaining modeling errors. Therefore, the simplest forms such as Monod are mostly chosen.

As for the function  $\pi$  related to product formation there may be several cases. If product formation is *growth associated* then  $\pi = k\mu$ , but the specific production rate can also be completely or partially independent of the specific growth rate. A typical model for partial dependence, found for instance in the case of lactic fermentation, is due to Luedekind and Piret [1]:

$$\pi = k\mu + \rho$$

where  $\rho$  is the non-growth associated specific production rate. If  $\pi$  is fully independent of  $\mu$ , then it will have a form similar to those seen above. In an



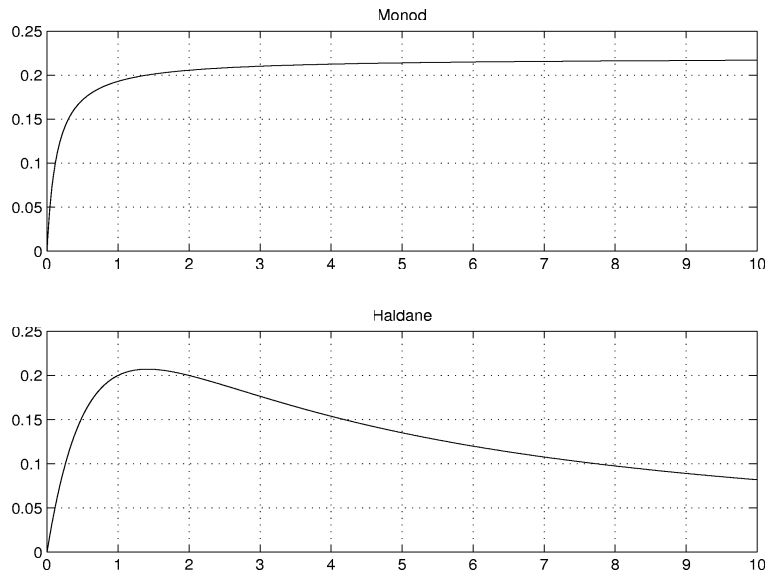


Fig. 2.2. Monod / Haldane kinetic functions

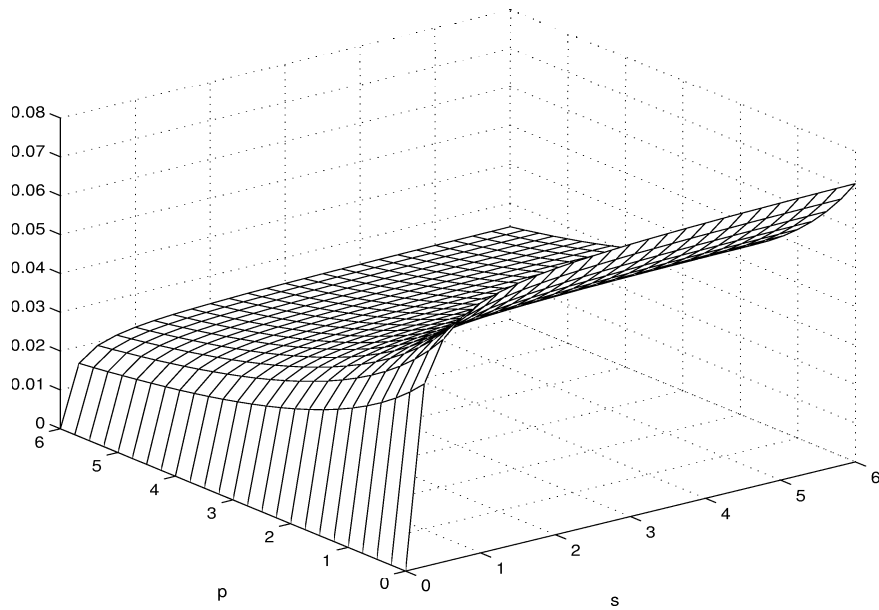


Fig. 2.3. Monod with product inhibition

alternative approach [145],  $\pi$  may be a nonlinear function of  $\mu$  or even its time derivative.

In [4] a classification of fermentations is given according to the form of  $\mu$  and  $\pi$ . Nothing is said about  $\sigma$ , but from previous examples it can be seen that in most cases it will be a function of the other two specific rates. According to this classification, there appear three main types:

- 1- Monotonic  $\mu$  and non-monotonic  $\pi$ . This type is most common, in which the cell growth is not inhibited or repressed while the product formation is inhibited. Fermentation of antibiotics such as penicillin and amino acids such as lysine belongs to this type.
- 2- Non-monotonic  $\mu$  and monotonic  $\pi$ . This type is less common. However, reports suggesting this may be the case, include glutamic acid fermentation on ethanol and vitamin B12 fermentation.
- 3- Non-monotonic  $\mu$  and non-monotonic  $\pi$ . This is the least common type. An example is ethanol fermentation from fructose.

For the multisubstrate case the kinetic functions suggested in the literature are generalizations of the previous ones. So, for example, in [146] the following examples are found:

- Multi-substrate Monod kinetics.

$$\mu = \mu_m \frac{s_1}{k_1 + s_1} \frac{s_2}{k_2 + s_2}$$

An example of such kinetics is the simultaneous requirement of glucose and oxygen by aerobically growing organisms.

- Double-Monod kinetics.

$$\mu = \mu_m \left( \frac{s_1}{k_1 + s_1} + \frac{s_2}{k_2 + s_2} \right) \left( \frac{1}{k_1 + k_2} \right)$$

An example of this kinetics is the parallel use of substrates, such as various types of sugars.

- Diauxic Monod growth.

$$\mu = \mu_{m1} \frac{s_1}{k_1 + s_1} + \mu_{m2} \frac{s_2}{k_2 + s_2 + s_1^2/k_I}$$

The consumption of substrate  $s_2$  will be inhibited until  $s_1$  is exhausted, for suitably low values of  $k_I$ . Diauxic growth can be observed in many organisms. An example is *E. coli*, where the uptake of lactose is repressed in the presence of glucose.

In [145], the following general structures are suggested:

- Interacting model for essential substrates.

$$\mu(s_1, s_2, \dots, s_n) = \mu_{max} r_1(s_1) r_2(s_2) \dots r_n(s_n)$$

– Non-interacting model for essential substrates.

$$\mu(s_1, s_2, \dots, s_n) = \min(\mu_1(s_1), \mu_2(s_2), \dots, \mu_n(s_n)) \quad \mu_i(s_i) = \mu_{imax} r_i(s_i)$$

– Model for growth-enhancing and alternative substrates.

$$\mu(s_1, s_2, \dots, s_n) = \mu_1(s_1) + \mu_2(s_2) + \dots + \mu_n(s_n) \quad \mu_i(s_i) = \mu_{imax} r_i(s_i)$$

As it has been stressed in this section, for control puposes, the relevant characteristics of the kinetic functions are

- Boundedness
- Monotonicity vs. non-monotonicity

In addition, the simplest forms are often chosen and in general they can be approximated by products/sums of relatively simple rational functions. In this case, systems are represented by sets of differential polynomial equations which are easier to handle with computer algebra systems such as Maple or Mathematica. This point is not considered here, but opens an interesting line for future research [150].

## 2.5 A catalogue

Finally, a list of typical models is shown below. It includes several examples of top industrial microorganisms. Some of the models are set for production in batch mode, i.e, with no input or output flux. Later on, a few examples of biphasic and other models are included.

- *Bacillus subtilis* [16], which corresponds to the second type model (see 2.9) with  $y_{sp} = 0$  and adds an hydrolisis constant  $k$ .

$$\begin{aligned} \dot{x} &= \mu x - Dx \\ \dot{s} &= -\mu x + D(s_i - s) \\ \dot{p} &= \pi x - kp - Dp \\ \dot{v} &= F \\ \mu &= \frac{\mu_{max} s}{k + s} \\ \pi &= \frac{k_p s}{K_p + s + K_i s^2} \end{aligned} \tag{2.19}$$

- *Corynebacterium* (L-glutamic acid production) [18], which exactly corresponds to the second type model.

$$\begin{aligned}
\dot{x} &= \mu x \\
\dot{s} &= -\frac{1}{y_{x/s}}\mu x - \frac{1}{y_{p/s}}\pi x \\
\dot{p} &= \pi x \\
\mu &= \frac{\mu_{max} s}{s + K_s(1 + \frac{p}{K_{ipx}})} \\
\pi &= \frac{\pi_{max} s}{K_{ps} + s(1 + \frac{s}{K_r})}
\end{aligned} \tag{2.20}$$

- *Candida parapsilosis* (Xylitol production) [20]. In this example oxygen is the limiting substrate. This model is of the first type (see 2.8).

$$\begin{aligned}
\dot{x} &= \mu x \\
\dot{s} &= -\frac{1}{y_{O_2}}\mu x + k_L a(s^* - s) \\
\dot{p} &= \alpha \mu x \\
\mu &= \frac{\mu_{max} s}{K_{O_2} + s}
\end{aligned} \tag{2.21}$$

- Microbiological Ethanol Synthesis by means of *Zymomous mobilis* [22]. An example of complex kinetic functions in which, as a curiosity,  $s_{in}$  enters in the expressions for  $\mu$  and  $\pi$ . It can be ascribed to the second type model with  $y_{sx} = 0$ .

$$\begin{aligned}
\dot{x} &= \mu x - Dx \\
\dot{s} &= -\frac{1}{Y_{p/s}}\pi x + D(s_{in} - s) \\
\dot{p} &= \pi x - Dp \\
\dot{v} &= F \\
\mu &= \frac{\mu_m s}{K_{1s} + s} \left(1 - \left(\frac{p}{p_{1m}}\right)^a\right) \left(1 - \frac{x}{x_m}\right)^2 \frac{K_i - s_{in}}{(K_i - s_{in}) - (s - s_{in})} \\
\pi &= \frac{q_{pm} s}{K_{2s} + s} \left(1 - \left(\frac{p - p_i}{p_{2m} - p_i}\right)^b\right) \left(\frac{K_i}{K_i + s}\right)
\end{aligned} \tag{2.22}$$

- Lactic fermentation [23] which corresponds to the more general model (see 2.10). The most common model for lactic fermentation found in the literature assumes  $\pi = constant$ , so it can be ascribed to the second type model.

$$\begin{aligned}
\dot{x} &= \mu x - k_d x - Dx \\
\dot{s} &= -\frac{1}{y_{x/s}}\mu x - \frac{1}{y_{p/s}}\pi x + D(s_i - s) \\
\dot{p} &= y_p \mu x + \pi x - Dp \\
\dot{v} &= F \\
\mu &= \frac{\mu_{max}s}{k+s} e^{-K_i p} \\
\pi &= \frac{\pi_{max}s}{k+s}
\end{aligned} \tag{2.23}$$

- Penicilline production [30] to [32]. This model also corresponds exactly to the second type model.

$$\begin{aligned}
\dot{x} &= \mu x - Dx \\
\dot{s} &= -\sigma x + D(s_i - s) \\
\dot{p} &= \pi x - kp - Dp \\
\dot{v} &= F'
\end{aligned} \tag{2.24}$$

$$\begin{aligned}
\mu &= \frac{0.11s}{0.006x + s} \\
\pi &= \frac{0.004s}{0.0001 + s + 10s^2} \\
\sigma &= \frac{\mu}{0.47} + \frac{\pi}{1.2} + 0.029 \\
k &= 0.01
\end{aligned}$$

- *Saccharomyces cerevisiae*. Here, several models are given depending on the operating conditions.

- a) Anaerobic fermentation on glucose [26]. This model corresponds to the second type model with  $y_{sp} = 0$ . Notice that  $\mu$  and  $\pi$  have the same form.

$$\begin{aligned}
\dot{x} &= \mu x - Dx \\
\dot{s} &= -\frac{1}{Y}\mu x + D(s_i - s) \\
\dot{p} &= \pi x - Dp \\
\dot{v} &= F' \\
\mu &= \frac{\mu_m s}{K + s} \frac{1}{1 + \frac{p}{K_p}} \\
\pi &= \frac{\pi_m s}{K' + s} \frac{1}{1 + \frac{p}{K'_p}}
\end{aligned} \tag{2.25}$$

- b) Aerobic fermentation on ethanol. This model, representing a particular recombinant strain (T73 BGL, see chapter 5), exactly corresponds to the first type model.

$$\begin{aligned}\dot{x} &= \mu x - Dx \\ \dot{s} &= -\frac{1}{Y}\mu x + D(s_i - s) \\ \dot{v} &= F \quad \mu = \frac{\mu_m s}{K + s}\end{aligned}\tag{2.26}$$

- *Kluyveromyces lactis*. Production of S-adenosyl-L-methionine. [147]. This model corresponds to a hybrid type, plus a time-dependent term suggested by the authors to account for a time lag and a maintenance coefficient  $m$ .

$$\begin{aligned}\dot{x} &= \mu x \quad \mu = \frac{\mu_m s}{K + s}(1 - e^{-t/t_L}) \\ \dot{s} &= -q_s x \quad q_s = \frac{\mu}{Y_{x/s} + m} \quad \text{Pirt - equation.} \\ \dot{p} &= q_p x \quad q_p = A\mu + B \quad \text{Luedekind - Piret.}\end{aligned}\tag{2.27}$$

Some key features, namely model type and influence of product on growth, are summarized in the following table.

example	model type			product
	I (2.8)	II (2.9)	hybrid (2.10)	
<i>Bacillus subtilis</i>		x		no
<i>Corynebacterium</i>		x		yes
<i>Candida parapsilosis</i>	x			no
<i>Zymomous mobilis</i>		x		yes
<i>Lactic fermentation</i>			x	yes
<i>Penicillin production</i>		x		no
<i>S. cerevisiae. Anaerobic</i>		x		yes
<i>S. cerevisiae. Aerobic</i>	x			no
<i>Kluyveromyces lactis</i>			x	no

As a complement to this section, some examples are given of unstructured non-segregated biphasic models. It must be noticed that, although more complex, they are at most of order six:

- *Saccharomyces cerevisiae*. Several models are given in [43] to [45].
- *Baker's yeast* [27] (akin to *S. cerevisiae*). The model is in absolute masses and considers gas exchange.

- One substrate plus limitation in oxygen. Penicillin production [146]. Constants  $k_1$  and  $k_2$  are turned on and off according to the value of  $\mu$ .
- Growth enhancing substrates. *P. vulgaris* on glucose and citric acid [145] (Tsao and Yang).
- *Escherichia coli* [37]. This example does not fall in any of the categories described in previous sections. The substrate  $s_2$  is an *inducer* which influences the production of  $p$  but does not affect growth directly. Its indirect influence on growth is due to the fact that  $p$  is inhibitory.

## 2.6 Structured and segregated models

Some phenomena, causing a variation in the biomass activity per unit biomass concentration, can not be properly handled by unstructured models. Examples are [146]:

- Loss of plasmids.
- Induction and repression of genes.
- Variation of RNA content of the cells.
- Variation of enzyme content of the cells.
- Post-translational modification of proteins.
- Signaling networks.
- Membrane transport.
- Accumulation of storage materials.
- Morphological changes, e.g. branching of filamentous organisms, volume to surface ratio of yeast cells.

Structured models provide information about the physiological state of the microorganisms, their composition and regulatory adaptation to the environment. Cell mass is structured into several intracellular compounds and functional groups, connected to each other and to the environment by fluxes of material and information. A structured model should normally be constructed of as few elements as possible. Otherwise the difficulties for the experimental model verification and parameter identification become insuperable [145]. Sometimes, even the verification of simple *unstructured* models is not possible owing to experimental difficulties. Moreover, getting on-line information of internal variables is impossible or extremely difficult due to sensorization and observability problems. For this reason structured models are seldom used for design or control [146]. Structured models may be useful to model transient behaviour of a biological system, caused for example by one or some of the phenomena in the list above, or if a wide range of changes of environmental conditions have to be described with one model and one set of parameters. In other words, structured (and/or segregated) models are mainly of interest for basic research. The complexity increases too much with no clear advantages for production and control purposes. On the other hand,

all the phenomena described above can not be plainly ignored and constitute one argument more favouring the design of robust controllers. Surveys on structured modelling can be found in [47] and [48]. Particular examples in [49], [50], [51] and [52]. For segregated models see [53] and [54].

## 2.7 Non-linearity and Uncertainty

As can be seen, these systems are in general highly nonlinear. The nonlinearities are most noticeable in the kinetic functions. These systems also show both parametric and unstructured uncertainty:

1. Dynamic (or unstructured) uncertainty is due to:
  - The use of unstructured and non-segregated models. Thus, ignoring part of the system dynamics which is *lumped* into some key factors. See the introduction and the previous section.
  - Besides the previous point, other assumptions such as homogeneity or some further model simplifications are usually made. Let's see as an example a simple case which only comprises the growth of biomass on a single substrate. The production is in *batch* mode, i.e. with no inputs or outputs (except for gas exchange). Then:

$$\begin{aligned}\dot{x} &= (\mu - k_d)x \\ \dot{s} &= -(y_s\mu + k_m)x \\ \dot{v} &= 0\end{aligned}\tag{2.28}$$

where  $x$  (g/l) is the biomass,  $s$  (g/l) substrate concentration and  $v$  volume. The parameters are,

- \*  $k_d$ : used to take into account the natural death of microorganisms or a loss of the division capacity. This term is usually disregarded.
- \*  $k_m$  is a *maintenance term* representing the amount of substrate used for the biomass survival. Usually, it is disregarded and its effect lumped into  $y_s$ , which becomes an apparent yield coefficient.

2. Parametric uncertainty may be due to:
  - Identification problems.
  - The fact that not two populations are equal, because of environmental effects, the preparation of the inoculus.....
  - Aging of cells which is reflected in slight variations of certain parameters during an experiment. For example, the yield coefficient. It is usually considered constant but with the aging of cellular population a greater quantity of substrate is needed to produce the same amount of biomass. Hence,  $y_s$  will tend to increase, i.e. there is a drift with time.
  - In general, any change in the environment or in the broth can potentially affect the system. Microorganisms are living things that continuously adapt themselves to changing conditions.



3. Uncertainty in the actuators (e.g. in the peristaltic pumps).

In addition to all these factors, the system is also affected by perturbations. The most important ones, not included in the model, are reflected in:

1. Volume, due for example to the samples for off-line analysis, the evaporation caused by aeration, etc.
2. In some cases the substrate concentration in the inflow may suffer variations.

## 2.8 Conclusions

In this chapter, it has been shown how a reduced number of models with relatively few state variables and a particular structure represent most applications. Moreover, even if multisubstrate and/or multiorganism systems were taken into account it would still be possible to work with relatively simple models with similar structures. Models for mixed cultures have not been considered but some examples can be found in [55] and [56]. Is there any point in considering some structured models? Although it may be interesting to explore this possibility in some particular instances, the increase in model complexity is seldom balanced by the gains in a finer description. Finally, other high density cultures of microorganisms such as fixed bed bioreactors or hollow fibre ones [145] have not been considered in this review.

The main characteristics of the process, relevant for control design, have been outlined. In the following chapters several key points are assumed:

1. All the work will be centred around the basic models described in section 2.3 for pure cultures with only one limiting substrate and assuming oxygen is in excess.
2. Only biomass and volume are measured on-line. No estimation of the specific rates is used. These demands are a consequence of practical considerations, but on the other hand it is also interesting in itself to analyze what can be done with a minimum of information.
3. The main control specification will be to keep a constant growth rate. This is usually associated by the biologists with the maintenance of a definite physiological state. It also appears in applications of optimal control to biotechnological processes. For references on this research area see [70], [71].
4. Three actuator configurations are to be used: 1) only the flux  $F$ , 2) both  $F$  and  $s_i$ , and finally 3)  $F$  and  $\alpha$ .

Other central factors, namely nonlinearity and uncertainty, determine the techniques to be used and the search for a robust/adaptive controller.



## 3 Invariant control

This chapter addresses the computation of invariant control laws. It will be seen how to derive partial state feedbacks that, assuming ideal conditions and perfect model, keep the specific growth rate  $\mu$  constant provided the initial conditions are adequate. These feedbacks depend on a reduced number of tunable parameters (at most two) which the stabilizing controllers designed in the next chapter modify suitably. The chapter begins with the simplest case, for which the invariant control law can be easily deduced from *physical* considerations. This law is the closed loop version of the *exponential feeding* already suggested in several references as shown later. It was also found that the closed loop version had been suggested previously in two references. But, there is *no* invariance and/or stability analysis. Moreover, the geometric approach taken here is new. This approach has led to the determination of other important objects for the designs in chapter 4. Mainly, the goal manifold to be followed by the system if  $\mu$  is to be kept constant. Also, the basic law is extended to more complex cases. Finally, in the second half of the chapter, a study of both local and global stability within the framework of partial stability is included.

### 3.1 Introduction

As it has been mentioned above, for the simpler case in which product is not considered as in

$$\begin{aligned}\dot{x} &= \mu(s)x - \frac{F}{v}x \\ \dot{s} &= -y\mu(s)x + \frac{F}{v}(s_i - s) \\ \dot{v} &= F\end{aligned}\tag{3.1}$$

an *exponential feeding law* has already been suggested. This law can be expressed as

$$F(t) = \lambda x(t)v(t) = \lambda x_0 v_0 e^{\mu_r t} \quad \text{for some } \lambda = \text{const.}\tag{3.2}$$

where  $x_0$  and  $v_0$  are the initial conditions for biomass and volume;  $\mu_r$  is the desired growth rate. For references see, in the 80's [80] and [81], also through the 90's [57], [59], [61], [62], [63], [65], [66], [67], [72], [77], [78] and recently [69], [73], [75], [76]. In a few references, [65], [66], [67] and [76], corrections are introduced modifying a constant ( $\lambda$ ,  $\mu_r$ ...) depending on the results of different measures.

Actually, law 3.2 can be deduced heuristically. There are two starting points:

- a) From past experience biotechnologists know that in order to keep a constant biomass growth rate in a fed-batch fermentation, feeding flux should "grow exponentially".
- b) Assume all individuals in a population can be identified with an average individual. Regard this individual as a processing unit that needs a determined quantity of energy so as to maintain a given level of activity. Then it follows that the quantity of substrate supplied, and consequently the feeding flux, must be proportional to the total population.

Designating the total mass population by  $\bar{x} = xv$ , it can be checked that  $\dot{\bar{x}} = \mu(s)\bar{x}$ . If  $\mu(s) = \mu_r$  then  $\dot{\bar{x}} = \mu_r\bar{x}$  and  $\bar{x}(t) = \bar{x}_0 e^{\mu_r t}$ . From b) it is immediate that for some constant  $\lambda$

$$F(t) = \lambda\bar{x}(t) = \lambda\bar{x}_0 e^{\mu_r t}$$

The use of a closed loop law, i.e. measuring on-line biomass and volume,

$$F = \lambda xv \quad \text{for some } \lambda = \text{const.} \quad (3.3)$$

is suggested for the first time in [64](1992). The difficulties in measuring biomass, at least for some ranges, are the probable cause for the loss of interest and only a similar law appears again in an academic article [100](1999), [101](2002). It is derived using complex techniques involving non-linear state-dependent time-scalings and applied in simulations to a fourth order baker's yeast fermentation process model. It is proved that law ensures the substrate concentration will be within a given range i.e. below a given  $s_{crit}$ . Other appearances are [125], [126] (2001) and [151](2003) in which experimental tests are shown. See also [74] (2003) for continuous bioreactors.

Finally, in adaptive control of (bio)chemical reactors [70] a similar law is used but needs the full state plus an estimation of an specific rate. In order to solve this problem, it is "approximated" taking the substrate concentration  $s$  equal to  $s_r$  a reference concentration.

A simple deduction of 3.3 could be as shown below. Consider the model 3.1. Notice the kinetic function  $\mu$  depends only on the substrate concentration. In order to keep a constant  $\mu(s)$ , the substrate concentration should be kept constant at a value  $s = s_r$  for which  $\mu(s_r) = \mu_r$ .

Taking the flow rate  $F = \lambda xv$ , the equation for substrate becomes

$$\dot{s} = (-y\mu(s) + \lambda(s_i - s))x$$

Provided  $s = s_r$  from the beginning, it is possible to keep  $\dot{s} = 0$  using

$$\lambda = \frac{y\mu_r}{s_i - s_r} = \text{constant} \quad (3.4)$$

independently of the initial conditions for  $x$  and  $v$ . In such a case, the trajectories followed by these two state variables would be defined by an “exosystem”

$$\begin{aligned} \dot{x} &= \mu_r x - \lambda x^2 \\ \dot{v} &= \lambda xv \end{aligned} \quad (3.5)$$

Conversely, if biomass  $x$  and volume  $v$  follow a trajectory defined by (3.4)-(3.5) then necessarily  $\dot{s} = 0$  and  $s = s_r$ . This is related to the fact that the system (3.1) is flat with flat outputs  $x$  and  $v$ . See for example [88] and next section.

In order to get explicit expressions for the manifold to be tracked, it must be noticed that the first equation in (3.5) is a logistic one, with solution:

$$x(t) = \frac{\frac{\mu_r}{\lambda}}{1 + \left(\frac{\mu_r}{\lambda x_0} - 1\right)e^{-\mu_r t}} \quad (3.6)$$

The volume trajectory is easily obtained after realizing that absolute mass  $\bar{x} = xv$  follows an exponential trajectory and  $\dot{v} = \lambda \bar{x}$ . Hence

$$v(t) = v_0 + \frac{\lambda x_0 v_0}{\mu_r} (e^{\mu_r t} - 1) \quad (3.7)$$

Solving for  $t$  and equating (3.6) and (3.7):

$$x - \frac{\mu_r}{\lambda} - \left(x_0 v_0 - \frac{\mu_r}{\lambda} v_0\right) \frac{1}{v} = 0 \quad (3.8)$$

which, along with

$$s - s_r = 0 \quad (3.9)$$

define a goal manifold, referred in the sequel as  $Z^*$ . This manifold, and in particular expression 3.8, will be very important in the developments of chapter 4.

### 3.2 Using flatness

In this section an alternative deduction of 3.3 based on flatness theory is presented.<sup>1</sup> Differentially flat systems are underdetermined systems of nonlinear differential equations whose solution curves are in a one-to-one smooth correspondence with a given set of arbitrary curves in a space of dimension equal to the number of equations by which the original system is underdetermined. For control systems this is equivalent to the number of control inputs. The components of the application from the system's space into the space of smaller dimension are called flat outputs. These outputs are, generally, a function of the dependent variables and a finite number of their derivatives. With them it is possible to parametrize the whole set of solutions of the ODE system without need of additional constants such as the initial conditions.

A general undetermined system of differential equations of order  $k$  can be written as

$$F^j(t, x, x^{(1)}, \dots, x^{(k)}) = 0, \quad j = 1, \dots, N - p \quad (3.10)$$

where it is assumed that  $F^j$  are  $C^\infty$  functions,  $x = (x_1, \dots, x_N)$  dependent variables,  $t$  independent variable,  $x^{(r)}$  the  $r$ -th derivative of  $x$  w.r.t time and  $p \geq 1$  is the number of equations by which the system is underdetermined.

System 3.10 is said to be differentially flat if there exist variables  $y^1, \dots, y^p$  (the *flat outputs*) given by equations of the form

$$y = h(t, x, x^{(1)}, \dots, x^{(m)}) \quad (3.11)$$

in such a way that the original variables  $x$  can be expressed in terms of  $y$  (locally) using an equation of the form

$$x = g(t, y, y^{(1)}, \dots, y^{(l)}) \quad (3.12)$$

In a particular case, the flat outputs only depend on the dependent variables and not on their derivatives. Then, we speak of *0-flatness*.

With these expressions it is possible to parametrize all the solutions of the original system of differential equations. There is no need of integrating the equations, instead we work with algebraic expressions.

In control theory the set of the dependent variables is divided into two sets: the states and the inputs. In addition, systems usually have the form:

---

<sup>1</sup> Further results based on this theory are found in the following chapter and in appendix A.

$$\dot{x} = f(x, u) \quad (3.13)$$

although there may be other representations. System (3.13) with  $m$  inputs  $u$  and  $n$  states  $x$  is said to be differentially flat if there exists a set  $m$  of variables

$$y_i = h_i(x, u, \dot{u}, \dots, u^{(\gamma_i)}) \quad i = 1, \dots, m.$$

such that

- (i) the  $m$  components of  $y$  are differentially independent, i.e., they are not related by any differential equation

$$Q(y, \dot{y}, \dots, y^{(\beta)}) = 0$$

- (ii) states  $x$  and inputs  $u$  can be expressed as functions of  $y$  and their derivatives in finite number:

$$\begin{aligned} x &= \Phi(y, \dots, y^{(\nu)}) \\ u &= \Psi(y, \dots, y^{(\nu+1)}) \end{aligned}$$

with  $\Phi$  and  $\Psi$  satisfying identically  $\dot{\Phi} = f(\Phi, \Psi)$ .

From the previous expressions it is easy to deduce that, if the trajectories to be followed by the flat outputs are fixed, it is possible to obtain the trajectories followed by the states and how the control actions in open loop should be.

The alternative deduction of 3.3 announced at the beginning of the section can be obtained realizing that system 3.1 is flat. Taking as inputs  $F = Dv$  and  $s_i$ , and as flat outputs  $x$  and  $v$  we have

- From the first and third equations in 3.1

$$s = \frac{k_s}{\frac{\mu_m x v}{x v + \dot{v} x} - 1} = \frac{k_s \dot{x}}{\mu_m \bar{x} - \dot{\bar{x}}} \quad (3.14)$$

- From the third equation

$$F = \dot{v} \quad (3.15)$$

- And from these and the second equation, it can be deduced the expression for  $s_i$  which will be a function of  $(x, v, \dot{x}, \dot{v}, \ddot{x}, \ddot{v})$ .

Consequently, if we force some specific trajectories of  $x$  and  $v$ , then  $s$  will go to the desired point. It is also easy to check that if the absolute quantity of biomass follows an exponential trajectory

$$\bar{x} = x_0 v_0 e^{\mu_r t} \quad (3.16)$$

then, substituting in (3.14),

$$s = \frac{k_s \mu_r}{\mu_m - \mu_r} \quad (3.17)$$

Which obviously coincides with the expression deduced previously. Now taking the expression for the volume  $v$  in  $Z^*$  and substituting in  $F = \dot{v}$  the exponential feeding law is obtained. This is also clear looking at the exosystem. The value of  $s_i$  could be obtained in a similar way. If the corresponding inputs are applied and the initial condition for the substrate concentration is such that  $s_0 = s_r$  then the system follows the desired trajectories and in fact the law can be used in closed loop, being an invariant control.

### 3.3 Invariance

Now, it must be confirmed that there exists an invariant control. That is, a control such that once the state is in  $Z^*$  (3.8 and 3.9), it stays there. The condition for  $(f, g)$ -invariance of system

$$\dot{\mathbf{x}} = f(\mathbf{x}) + g(\mathbf{x})u$$

with respect to the submanifold  $Z^*$  implies the existence of a solution  $u = u(\mathbf{x})$  (the invariant control) of the algebraic equation

$$\frac{\partial \varphi}{\partial \mathbf{x}} f(\mathbf{x}) + \frac{\partial \varphi}{\partial \mathbf{x}} g(\mathbf{x})u(\mathbf{x}) = 0 \quad \mathbf{x} \in Z^* \quad (3.18)$$

where  $\varphi$  is a vector containing the expressions in the equations 3.8-3.9 defining  $Z^*$ . In our case the control law is defined by the partial state feedback:

$$u(\mathbf{x}) = \lambda x v \quad (3.19)$$

Two equations are obtained, the first one is fulfilled for every  $\lambda$ . The second one gives

$$\lambda = \frac{y \mu_r}{s_i - s_r} \quad (3.20)$$

as it was expected.



This result can be extended to processes in which the equation corresponding to a product is added. Let us introduce a new control action  $\alpha$ , representing a filtering of the broth:

$$\begin{aligned} \dot{x} &= \mu(s, p)x - \frac{F}{v}x \\ \dot{s} &= -y_{sx}\mu(s, p)x - y_{sp}\nu(s, p)x + \frac{F}{v}(s_i - s) \\ \dot{p} &= \nu(s, p)x - \frac{\alpha}{v}p - \frac{F}{v}p \\ \dot{v} &= F \end{aligned} \tag{3.21}$$

In this case, as it can be easily checked, the invariant control is:

$$\begin{aligned} F &= \lambda xv \\ \alpha &= \alpha' xv \end{aligned} \tag{3.22}$$

where  $\lambda$  and  $\alpha'$  are appropriate constants and the invariant manifold is defined by (3.8), (3.9) and

$$p - p_r = 0 \quad p_r = \text{constant}. \tag{3.23}$$

This specification being justified by the need of keeping the microorganism in a given physiological state, in which production of a specific metabolite is optimum. Product  $p$  may be an inhibitor and/or the metabolite of interest. The specification of  $p = \text{constant}$ , in turn, forces the introduction of the new control action  $\alpha$ .

### 3.4 Partial stability

Partial stability is defined as the stability of dynamic systems with respect *not* to all but just to a given part of the state variables [92]. It arises in the study of electromagnetics, inertial navigation systems, spacecraft stabilization via gimbaled gyroscopes and/or flywheels, vibrations in rotating machinery, biocenology ... see the references in [95]. See also [93] for a list of general situations leading to the investigation of partial stability problems.

The general definition given above, actually encompasses two different cases [93]:

- The Lyapunov-Rumyantsev PSt-problem.

Let there be given a nonlinear system of ordinary differential equations of perturbed motion

$$\dot{x} = X(t, x) \quad X(t, 0) = 0 \quad (3.24)$$

The variables constituting the phase vector of system 3.24 are divided into two groups  $x^T = (y^T, z^T)^T$ :

- the  $y$ -variables with respect to which the stability of the unperturbed motion  $x = 0$  is to be investigated.
- the remaining  $z$ -variables.

The unperturbed motion  $x = 0$  of system 3.24 is said to be  $y$ -stable, if for any numbers  $\epsilon > 0$ ,  $t_0 \geq 0$ , there is a number  $\delta(\epsilon, t_0) > 0$  such that if  $\|x_0\| < \delta$  then  $\|y(t; t_0, x_0)\| < \epsilon$  for all  $t > t_0$ .

- Stability of *partial* equilibrium positions.

Let there be given a nonlinear system of ordinary differential equations:

$$\dot{y} = Y(t, y, z) \quad \dot{z} = Z(t, y, z) \quad Y(t, 0, z) \equiv 0 \quad (3.25)$$

The set  $y = 0$  of system 3.25 is said to be stable, if for any numbers  $\epsilon > 0$ ,  $t_0 \geq 0$ , there is a number  $\delta(\epsilon, t_0) > 0$  such that from  $\|y_0\| < \delta$ ,  $\|z_0\| < \infty$  it follows that  $\|y(t; t_0, x_0)\| < \epsilon$  for all  $t > t_0$ .

This is the PSt-problem w.r.t. that part of the variables of the original system (or the corresponding perturbed motion system) for which this system has an equilibrium position. *Partial* equilibrium positions of this kind (also termed a *balanced motion*) are invariant sets of the system. Hence, it is actually the problem of stability of sets that is analyzed in this case.

Fed-batch bioreactors fit in the second case as shown later. In the first subsection a local analysis is carried out using the techniques in [134]. In the final subsection, a global analysis is carried out following [95]. In both subsections the same model used previously is assumed. The stability of the invariant control for the case with product is not dealt with and remains an open question. An attempt was made at extending the global analysis of subsection 3.4.2 to this case but no valid Lyapunov function has been found for the time being. Going back to our main point, it must be noted that although the local analysis only deals with systems with Monod kinetics, the global analysis considers both Monod and Haldane kinetic functions.

### 3.4.1 Local analysis.

The main goal of this subsection is to check whether the invariant manifold is (at least) locally stable. With this purpose in mind, a new coordinate system is obtained that has one coordinate  $z$  along  $Z^*$  and two other coordinates  $\xi_1, \xi_2$  transversal to it. The system model transforms into a *task-oriented model* [94], [134], which will be linearized with respect to  $\xi_1, \xi_2$ . The procedure only gives local results, but on the other hand it is very systematic.

The new coordinates can be defined by  $\xi = \varphi(x, s, v)$  and a mapping from  $Z^*$  to  $Z$ , i.e.:

$$\begin{aligned}
\xi_1 &= x - \frac{\mu_r}{\lambda} - (x_0 v_0 - \frac{\mu_r}{\lambda} v_0) \frac{1}{v} \\
\xi_2 &= s - s_r \\
z &= xv
\end{aligned} \tag{3.26}$$

The inverse transformation is given by:

$$\begin{aligned}
x &= \frac{z(\xi_1 + \frac{\mu_r}{\lambda})}{z - (x_0 v_0 - \frac{\mu_r}{\lambda} v_0)} \\
s &= \xi_2 + s_r \\
v &= \frac{z - (x_0 v_0 - \frac{\mu_r}{\lambda} v_0)}{(\xi_1 + \frac{\mu_r}{\lambda})}
\end{aligned} \tag{3.27}$$

For the sake of simplicity define

$$\begin{aligned}
b &\triangleq \frac{\mu_r}{\lambda} \\
a &\triangleq x_0 v_0 - \frac{\mu_r}{\lambda} v_0
\end{aligned}$$

The resulting *task-oriented model* is

$$\begin{aligned}
\dot{\xi}_1 &= \mu(\xi_2 + s_r) \frac{z(\xi_1 + b)}{z - a} - \frac{(\xi_1 + b)^2}{z - a} u \\
\dot{\xi}_2 &= -y\mu(\xi_2 + s_r) \frac{z(\xi_1 + b)}{z - a} + \frac{(\xi_1 + b)(s_i - s_r - \xi_2)}{z - a} u \\
\dot{z} &= \mu(\xi_2 + s_r)z
\end{aligned} \tag{3.28}$$

A control law may be defined as

$$u = U_\xi(z) + \bar{u} = \lambda z + \bar{u} \tag{3.29}$$

where  $U_\xi(z)$  is the invariant control in the new coordinates, and  $\bar{u}$  a stabilizing control to be designed in case  $Z^*$  is not stable only with  $U_\xi(z)$  (or to try to improve performance).

From this point onwards, it is necessary to assume a definite form for  $\mu$ . In this case, a Monod function. The system to be linearized with respect to the  $\xi$  coordinates is

$$\dot{\xi} = \bar{f}_\xi(\xi, z) + g_\xi(\xi, z)\bar{u}$$

where

$$\overline{f_\xi} = f_\xi + g_\xi U_\xi \quad \overline{f_\xi}(0, z) = 0$$

with

$$f_{\xi_1} = \mu(\xi_2 + s_r) \frac{z(\xi_1 + b)}{z - a}$$

$$f_{\xi_2} = -y\mu(\xi_2 + s_r) \frac{z(\xi_1 + b)}{z - a}$$

and

$$g_{\xi_1} = -\frac{(\xi_1 + b)^2}{z - a}$$

$$g_{\xi_2} = \frac{(\xi_1 + b)(s_i - s_r - \xi_2)}{z - a}$$

The resulting linear system has the form

$$\dot{\xi} = A(z)\xi + b(z)\bar{u} \quad A = \frac{\partial \overline{f_\xi}}{\partial \xi} \Big|_{\xi=0} \quad b = g(0, z)$$

and the complete expressions assuming Monod kinetics

$$\begin{aligned} \dot{\xi}_1 &= \left( \frac{\mu_r z}{z - a} - \frac{2b}{z - a} \lambda z \right) \xi_1 + \\ &\quad + \left( \frac{\mu_m k}{(k + s_r)^2} \frac{zb}{z - a} \right) \xi_2 - \frac{b^2}{z - a} \bar{u} \\ \dot{\xi}_2 &= \left( -y \frac{\mu_r z}{z - a} + \frac{s_i - s_r}{z - a} \lambda z \right) \xi_1 + \\ &\quad + \left( \frac{-y\mu_m k}{(k + s_r)^2} \frac{zb}{z - a} - \frac{b}{z - a} \lambda z \right) \xi_2 + \frac{b(s_i - s_r)}{z - a} \bar{u} \end{aligned} \tag{3.30}$$

Define:

$$d \triangleq \frac{\mu_m k}{(k + s_r)^2}$$

It turns out that the A-matrix

$$A(z) = \frac{z}{z - a} \begin{bmatrix} -\mu_r & db \\ 0 & -ydb - \mu_r \end{bmatrix} \tag{3.31}$$

has both poles stable, with values:

$$\begin{aligned} p_1 &= -\frac{z}{z-a}(ydb + \mu_r) \\ p_2 &= -\frac{z}{z-a}\mu_r \end{aligned} \quad (3.32)$$

Hence the invariant manifold is (at least locally) stable.

As it has been mentioned in the introduction, this analysis is local and valid only for systems with Monod-like kinetic functions. In contrast, the analysis of the following subsection is global and valid for both Monod-like and Haldane-like functions. Nevertheless, the local analysis is still worthy since it provides a geometric view of the problem and some of the objects found in the process, namely the off-the-manifold variables  $\xi_i$ , will be used in the following chapter.

### 3.4.2 Global analysis

Assume a growth-linked type fed-batch bioreactor without inhibitor product, i.e. model 3.1. Now, in order to introduce the theorem to be used in the stability proof, consider the nonlinear autonomous dynamical system

$$\begin{aligned} \dot{x}_1 &= f_1(x_1, x_2), & x_1(0) &= x_{10}, & t &\in I_{x_0} \\ \dot{x}_2 &= f_2(x_1, x_2), & x_2(0) &= x_{20} \end{aligned} \quad (3.33)$$

where  $x_1 \in D \subseteq R^{n_1}$ ,  $D$  is an open set with  $0 \in D$ ,  $x_2 \in R^{n_2}$  and

$$f_1 : D \times R^{n_2} \longrightarrow R^{n_1}$$

is such that  $\forall x_2 \in R^{n_2}$

$$f_1(0, x_2) = 0.$$

and  $f_1(\cdot, x_2)$  is locally lipschitz in  $x_1$ . Also

$$f_2 : D \times R^{n_2} \longrightarrow R^{n_2}$$

is such that for every  $x_1 \in D$ ,  $f_2(x_1, \cdot)$  is locally lipschitz in  $x_2$  and  $I_{x_0} \triangleq [0, \tau_{x_0})$ ,  $0 < \tau_{x_0} \leq \infty$ , is the maximal interval of existence for the solution  $(x_1(t), x_2(t))$ ,  $t \in I_{x_0}$ . Under the above assumptions the solution exists and is unique over  $I_{x_0}$ .

Stability with respect to  $x_1$  of the system defined above can be defined as:

*The nonlinear dynamical system 3.33 is Lyapunov stable w.r.t.  $x_1$  if, for every  $\epsilon > 0$  and  $x_{20} \in R^{n_2}$ , there exists  $\delta(\epsilon, x_{20}) > 0$  such that  $\|x_{10}\| < \delta$  implies that  $\|x_1\| < \epsilon$  for all  $t \geq 0$ .*

Definition which corresponds with the notion of partial equilibria. In [95] there are also definitions for asymptotic stability and other. In order to analyze partial stability, the following results are used [95]:

**Theorem 3.1.** Consider system 3.33, then if there exists a continuously differentiable function  $V : D \times R^{n_2} \mapsto R$  and a class  $k$  function  $\alpha(\cdot)$  such that

$$\begin{aligned} V(0, x_2) &= 0 \quad x_2 \in R^{n_2} \\ \alpha(\|x_1\|) &\leq V(x_1, x_2) \quad (x_1, x_2) \in D \times R^{n_2} \\ \dot{V}(x_1, x_2) &\leq 0 \quad (x_1, x_2) \in D \times R^{n_2} \end{aligned}$$

Then system 3.33 is Lyapunov stable with respect to  $x_1$ .

**Corollary 3.2.** Consider system 3.33. If there exists a continuously differentiable, positive definite function  $V : D \mapsto R$  such that

$$V'(x_1)f_1(x_1, x_2) \leq 0, \quad (x_1, x_2) \in D \times R^{n_2}$$

then system 3.33 is Lyapunov stable with respect to  $x_1$ , uniformly in  $x_2$ . If in addition there exists a class  $K$  function  $\gamma(\cdot)$  such that

$$V'(x_1)f_1(x_1, x_2) \leq -\gamma(\|x_1\|), \quad (x_1, x_2) \in D \times R^{n_2}$$

then system 3.33 is asymptotically stable w.r.t  $x_1$ , uniformly in  $x_2$ .

*Proof.* Direct consequence of theorem 1 with  $V(x_1, x_2)$  replaced by  $V(x_1)^2$

Now consider

$$\begin{aligned} f_1 : \dot{s} &= (-y\mu(s) + \lambda(s_i - s))x \quad \text{with} \quad \lambda = \frac{y\mu_r}{s_i - s_r} \\ f_2 : \dot{x} &= \mu(s)x - \lambda x^2 \\ \dot{v} &= \lambda xv \end{aligned}$$

whenever  $s = s_r$  ( $\xi_2 = s - s_r = 0$ ), we have

$$f_1 = 0 \quad \forall x_2 = (x, v)$$

A candidate Lyapunov function is

$$V(x_1) = \frac{1}{2}(s - s_r)^2$$

It's derivative is

$$\begin{aligned} \dot{V} &= (s - s_r)\dot{s} \\ &= (s - s_r)(-y\mu(s)x + D(s_i - s)) \\ &= (s - s_r)(-y\mu(s)x + \frac{F}{v}(s_i - s)) \end{aligned}$$

<sup>2</sup> The symbol  $V'(\cdot)$  represents the Frechet derivative of  $V$  at  $x$  and in the previous theorem  $\dot{V}(x_1, x_2) \triangleq V'(x_1, x_2)f(x_1, x_2)$ . Informally, a Frechet derivative is a derivative defined for mappings from one vector space to another. See, for example, [153].

and taking  $F = \lambda xv$  it becomes

$$\dot{V} = (s - s_r)(-y\mu(s)x + \lambda x(s_i - s))$$

Assuming perfect knowledge of model parameters,

$$\lambda = \frac{y\mu_r}{s_i - s_r}$$

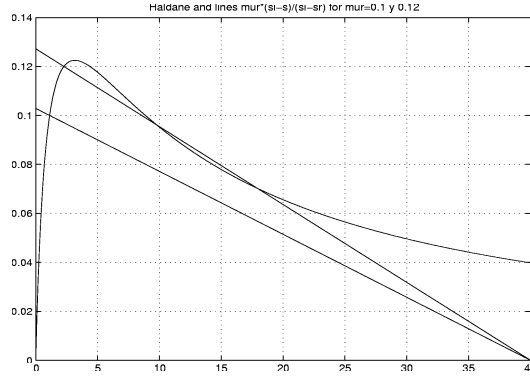
Hence,

$$\dot{V} = (s - s_r)(-y\mu(s)x + \frac{y\mu_r}{s_i - s_r}x(s_i - s))$$

or

$$\dot{V} = yx(s - s_r)(-\mu(s) + \mu_r \frac{s_i - s}{s_i - s_r}) \quad (3.34)$$

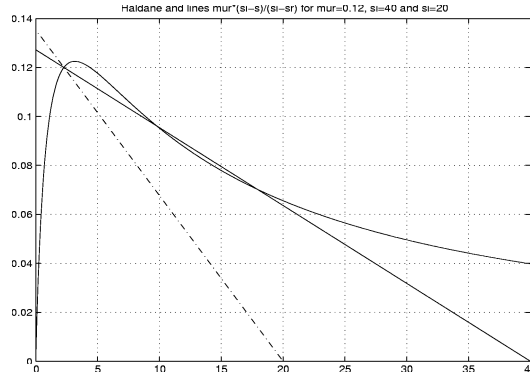
with  $y = \text{const} > 0$  and  $x > 0$ . Clearly, whenever  $s - s_r > 0$  the curve defined by  $\mu(s)$  must be over the straight line defined by  $\mu_r(s_i - s)/(s_i - s_r)$  and viceversa. See figure 3.1. This always happens whenever the kinetics function is monotonous or Monod-like, whereas in the Haldane-like case the parameter  $s_i$  may have to be chosen properly. See figure 3.2.



**Fig. 3.1.** Haldane and lines  $\mu_r(s_i - s)/(s_i - s_r)$  for  $\mu_r = 0.1, 0.12$

A more formal proof can be set up as follows. By the mean value theorem,

$$\mu(s) = \mu(s_r) + \frac{\partial \mu}{\partial s} \Big|_{\bar{s} \in [s_r, s]}(s - s_r)$$



**Fig. 3.2.** Haldane and lines  $\mu_r(s_i - s)/(s_i - s_r)$  for  $\mu_r = 0.12$  and  $s_i = 40, 20$

Now, defining a new variable  $\nu$  as  $\nu = (s - s_r)$  and substituting the previous expression into 3.34,

$$\dot{V} = -yx\left(\frac{\partial\mu}{\partial s}\Big|_{\bar{s}} + \frac{\mu_r}{s_i - s_r}\right)\nu^2 \quad (3.35)$$

In the Monod-like case the derivative of  $\mu$  will always be positive and hence the system will be stable. In the Haldane-like case the derivative may be, for some values of the substrate concentration  $s$ , negative and greater than the term  $\mu_r/(s_i - s_r)$  hence the system may be only locally stable. This proof can also provide some extra insight into the system performance. Since the Lyapunov function can be expressed as

$$V = \frac{1}{2}\nu^2$$

the expression 3.35 becomes

$$\dot{V} = -2yx\left(\frac{\partial\mu}{\partial s}\Big|_{\bar{s}} + \frac{\mu_r}{s_i - s_r}\right)V$$

Assuming the system is stable we have

$$m < \left(\frac{\partial\mu}{\partial s}\Big|_{\bar{s}} + \frac{\mu_r}{s_i - s_r}\right) < M$$

for some constants  $m$  and  $M$  both positive. Consequently the evolution of the function  $V$  is bounded by two functions. These depend on the value of the biomass concentration which varies with time, but we can still check that as the value of  $x$  increases the convergence is faster.



This result will be used in the following chapter to ensure stability of the closed loop imposing a saturation on the control action. In particular, imagine a controller in the form  $F = (\lambda_{nominal} + u)xv$  is available that works for systems with monod-like kinetic functions, i.e. monotonously increasing. Its applicability could be extended to systems with Haldane-like functions by means of a saturation on  $\lambda$ . This saturation would force the system to evolve towards the monotonously increasing part of the kinetic function. A simple method for choosing this saturation may be as follows: a maximum  $\lambda$  could be chosen to be that one corresponding with  $\mu_{max}$  and consequently with an  $s = s_{crit}$ . In a second step  $s_i$  should be chosen small enough to fulfil the condition above. If, for practical questions, were advisable to choose a bigger influx substrate concentration, then a smaller  $\lambda_{max}$  could be chosen as far as  $\lambda_{max} > \lambda_r$ .

Similar reasonings could be applied if it were desirable to stabilize the system around a point in the monotonously decreasing side of Haldane-like function. In this case it would be necessary to determine a  $\lambda_{min}$  value.

Finally, the result presented in this section could also be useful in analyzing already existing controllers. For example, in [154] a modification is presented of a controller suggested in [69] using an estimation of  $\mu$ . For system 3.1 this modification has the form:

$$F = \frac{y\mu}{s_i - s_r}xv - k \frac{y(\mu - \mu_r)}{s_i - s_r}xv$$

for some constant  $k$  to be chosen. This can be easily rewritten as

$$F = \frac{y\mu_r}{s_i - s_r}xv + (1 - k) \frac{y(\mu - \mu_r)}{s_i - s_r}xv$$

i.e. the invariant control plus the correction using the error in  $\mu$  multiplied by a new constant. Since the specific growth rate is bounded, there is implicitly a limitation on  $\lambda$ . Hence, for a properly chosen  $k$  we can make sure the system will always be in a given region. Actually, in [154] it is proved by other means the system can be globally stabilized at any desired setpoint  $s = s_r$  all along the (non-monotonic) kinetics.

### 3.5 Conclusions

In this chapter, an invariant control has been suggested that will provide the basis for subsequent designs. In particular, it will be a part of the controllers presented in the following chapter and the geometric approach in analyzing this law has supplied new coordinates useful in defining an *off-the-manifold* error variable. Besides, a local stability proof was obtained that encouraged

the search for a global one. This is provided in the final section of the chapter and will enable the treatment of those problems in which Haldane-like kinetic functions are present.

## 4 Dealing with uncertainty

### 4.1 Introduction

The results obtained in the previous chapter are essential for the developments to be presented here. Using a saturation on  $\lambda$ , it is possible to work with the monotonously increasing part of the kinetic functions. Consequently, and with no loss of generality, it is possible to work with the following model

$$\begin{aligned} \dot{x} &= \mu(s)x - Dx \\ \dot{s} &= -y\mu(s)x + D(s_i - s) \\ \dot{v} &= Dv \quad D = \lambda x \\ \mu(s) &\longrightarrow \textit{Monod-like} \end{aligned} \tag{4.1}$$

the results presented in the following sections being easily extended to the case in which  $\mu$  is Haldane-like. Additionally, in the conclusions some guidelines are given to extend these results to more complex systems including those in which a product must be considered. For system 4.1 three possible solutions are suggested:

1. One based on flatness and using two PIs.
2. A design based on a geometric approach [94], [134].
3. Finally, a robust adaptive controller.

In all these cases it will be necessary to make sure the system does not get stuck in the saturation.

### 4.2 PI controllers. Flatness

The concept of flatness, along with one of its applications, was presented in chapter 3<sup>1</sup>. In this section those results are used to design a simple controller consisting of the invariant control plus two PIs. These PIs take as error signal the difference between the actual measurements of the flat outputs  $x, v$  and their reference trajectories generated by an exosystem.

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<sup>1</sup> See also appendix A for an extensive survey on the subject

### 4.2.1 Controller design

Assume a model such as 4.1. Remember from previous sections the goal is

$$\mu = \mu_r = \text{constant} \quad (4.2)$$

Given that the model is supposed to be perfect and there are no perturbations, this goal could be achieved using the partial state feedback

$$F = \lambda xv \quad \lambda = \frac{y_s \mu_r}{s_i - s_r} \quad (4.3)$$

As explained in chapter 3, substituting in the model with concentrations:

$$\begin{aligned} \dot{x} &= \mu x - \lambda x^2 \\ \dot{s} &= (-y_s \mu + \lambda(s_i - s))x \\ \dot{v} &= \lambda x v \end{aligned} \quad (4.4)$$

Now, to overcome model uncertainty and the presence of perturbations, a correction on the basic control law must be introduced.

As commented in chapter 2, it is interesting to use both  $F$  (or equivalently  $\lambda$ ) and  $s_i$  as control actions, although it appears a coupling between the new control actions  $u_1 \triangleq \lambda$  and  $u_2 \triangleq s_i$ :

$$\begin{aligned} \dot{x} &= \mu x - u_1 x^2 \\ \dot{s} &= (-y_s \mu + u_1(u_2 - s))x \\ \dot{v} &= u_1 x v \end{aligned} \quad (4.5)$$

Since it is assumed there is not an online measure of the substrate concentration  $s$  in the bioreactor, an exponential trajectory for the absolute quantity of biomass  $\bar{x}$  will have to be forced. Remember that

$$\dot{\bar{x}} = \mu(s)\bar{x} \quad (4.6)$$

Given that  $\bar{x} = xv$ , this goal can be achieved forcing some specific trajectories on  $x$  and  $v$ . In order to generate these reference trajectories an exosystem is used:

$$\begin{aligned} \dot{x}_r &= \mu_r x_r - \lambda_n x_r^2 \\ \dot{v}_r &= \lambda_n x_r v_r \end{aligned} \quad (4.7)$$

with initial conditions  $x_{r0}$  and  $v_{r0}$ , being  $\lambda_n$  a nominal  $u_1$  determined using the expression in (4.3) given a nominal concentration  $s_{i_n}$  chosen a priori.

So, a multivariable non-affine system (4.5) with two inputs and two outputs is obtained. In the sequel two separate control loops are designed without taking into account the coupling between  $u_1$  and  $u_2$ .

With this goal in mind, and considering what has been previously said in chapter 2 about the effects of changing the input flux and substrate concentration in it, the following associations are made:

- $u_1$  with volume  $v$ .
- $u_2$  with biomass concentration  $x$ .

This association between inputs and outputs has an additional advantage. If the expression for  $\lambda_n$  in (4.3) is observed, it is easy to check that a variation in the yield coefficient  $y_s$  can be compensated changing  $u_2$  (i.e.  $s_i$ ), the value of  $u_1$  (i.e.  $\lambda$ ) remaining the same. Hence, the system will be to some extent robust against changes in the yield coefficients.

Volume can be easily controlled using a law of the form:

$$u_1 = \lambda_n + k_{p1}(e_v + \frac{1}{T_{I1}} \int e_v dt) \quad (4.8)$$

$$e_v = v_r - v$$

As for the biomass concentration, if the specific growth rate  $\mu$  is a monotonous function of the substrate concentration  $s$  then it will suffice with a similar law:

$$u_2 = s_{in} + k_{p2}(e_x + \frac{1}{T_{I2}} \int e_x dt) \quad (4.9)$$

$$e_x = x_r - x$$

since if  $e_x > 0$  ( $e_x < 0$ ) it is necessary to increase (decrease)  $\mu$  and consequently increase (decrease)  $u_2$ . The problem is more complex if functions analogous to the *Haldane* expression (fig. 2.2) are considered. In this case there exists a value  $s^*$  such that for  $s > s^*$  control action  $u_2$  must have a reversed sign, i.e. decreasing  $u_2$  increases  $\mu$ . Since dependence on the knowledge of  $s^*$  is critical, the effect of uncertainty on the control will be bigger. It would also be possible to restrict the operating zone to the monotonously increasing part of the kinetic function as indicated in the introduction. In this case a saturation on  $u_2$  would also be necessary. Simulated results are shown in chapter 5.

### 4.3 A nonlinear P controller

This section assumes again a model in the form 4.1. The data obtained in a real experiment are offered in chapter 5. The results presented here are strongly based on those of chapter 3. Namely:

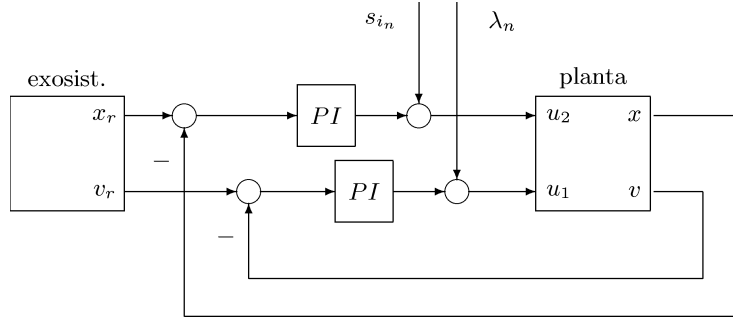


Fig. 4.1. Closed loop diagram for section 4.2

The definition of an invariant control

$$F = \lambda x v \quad \text{with} \quad \lambda = \frac{y \mu_r}{s_i - s_r} = \text{constant} \quad (4.10)$$

and the associated *goal manifold*  $Z^*$  to be tracked.

$$\begin{aligned} x - \frac{\mu_r}{\lambda} - (x_0 v_0 - \frac{\mu_r}{\lambda} v_0) \frac{1}{v} &= 0 \\ s - s_r &= 0 \end{aligned} \quad (4.11)$$

Based on the first two points, the computation of a task oriented model (eq. 3.28) which is linearized with respect to the  $\xi_i$  variables

$$\dot{\xi} = A(z)\xi + b(z)\bar{u} \quad A = \frac{\partial \bar{f}_\xi}{\partial \xi} \Big|_{\xi=0} \quad b = g(0, z) \quad (4.12)$$

where  $\bar{u}$  is an stabilizing control and the control action applied to the original system is

$$u = U_\xi(z) + \bar{u} = \lambda z + \bar{u} \quad (4.13)$$

For the partial linearization, it has been assumed that  $\mu$  is Monod-like.

Although the invariant control proved to be globally stable, it is necessary to take into account model imperfections and perturbations. Besides trying to see if performance can be improved. For this purpose, an additional proportional controller was designed.

### 4.3.1 Controller design

From the beginning, it was decided to design in the first place a proportional control law modifying the gain  $\lambda$  so as to obtain a simple controller in the form suggested at the end of the previous chapter. Since in addition, only biomass and volume are measured the controller  $\bar{u}$  was chosen

$$\bar{u} = k_p z \xi_1 \quad (4.14)$$

Following [94], if  $\bar{u}$  is chosen in the form

$$\bar{u} = k(z)\xi$$

where

$$k(z) = -b^T(z)P(z)$$

then the matrix  $P = P^T$  must be a solution to the algebraic Riccati equation

$$A^T(z)P(z) + P(z)A(z) - P(z)b(z)b^T P(z) = 2\eta(z)P(z)$$

where the function  $\eta(z)$  must satisfy the inequality

$$\eta(z) \leq \min_i \operatorname{Re} \lambda_i \{A(z)\} - \Delta,$$

for  $\Delta > 0$  is a small number.

The solution, obtained with a symbolic math program, implies that

$$k_p = 0$$

So, supposing a perfect model, the invariant control alone is the best option. But in a real situation some correction of the nominal invariant control action is necessary. As it can be seen in the experimental data in the following chapter, proportional action is not enough since there appears a steady state error in  $\mu$ . Hence some integral action is necessary.

## 4.4 A robust-adaptive controller

A robust adaptive controller is presented in this section. It is applied to processes with Monod-like and Haldane-like kinetic functions depending only on the substrate concentration. In the latter case, the results in chapter 3 may be used to determine a saturation in the control action to ensure stability. In the following subsection, the theoretical derivation of the controller is shown.

#### 4.4.1 Controller design

Consider model 4.1. The kinetics function may be monotonic or non-monotonic. Again, the basis for the new controller is the invariant control of chapter 3

$$F = \lambda xv$$

The goal manifold associated to it, also plays an important role. In fact, a new error variable is taken in the form

$$\sigma = \sigma(z, v, \lambda) = \frac{z - z_{or}}{z} - \frac{\mu_r}{\lambda} \frac{v - v_{or}}{z}$$

Notice that  $\sigma = \xi_1/x$ , i.e. the measurable off-the-manifold variable normalized by the concentration of biomass, in such a way that the errors are more important at the beginning of the fermentation rather than at the end. Here,  $\lambda$  is not taken as a constant but as a variable and  $z = xv$  being  $x_{or}, v_{or}, z_{or}$  the initial conditions for a reference trajectory which can be generated by exosystem

$$\begin{aligned}\dot{x} &= \mu_r x - \lambda x^2 \\ \dot{v} &= \lambda xv \\ \lambda &= \lambda_{nom} = \frac{y\mu_r}{s_i - s_r}\end{aligned}$$

In the sequel a law modifying  $\lambda$  is sought in order to compensate the effect of uncertainty. Consider the function

$$W = \frac{1}{2}\sigma^2 \tag{4.15}$$

It is intended to achieve  $\sigma \rightarrow 0$ . This could be achieved forcing

$$\dot{\sigma} = -\frac{1}{T_a}\sigma \tag{4.16}$$

so that

$$\dot{W} = -\frac{1}{T_a}\sigma^2 \leq 0 \tag{4.17}$$

The derivative of  $\sigma$  with respect to time is



$$\dot{\sigma} = \mu(s)(1 - \sigma) - \mu_r + \dot{\lambda} \frac{\mu_r(v - v_{or})}{\lambda^2 z} \quad (4.18)$$

From the equation 4.16 and solving for the derivative of  $\lambda$ :

$$\dot{\lambda} = \frac{\lambda^2 z}{\mu_r(v - v_{or})} [\mu_r - \mu(s)(1 - \sigma) - \frac{1}{T_a} \sigma] \quad (4.19)$$

Notice that if  $\sigma = 0$  then

$$\dot{\lambda} = \frac{\lambda^2 z}{\mu_r(v - v_{or})} [\mu_r - \mu(s)] \quad (4.20)$$

In case an adequate on-line measure or estimation of  $\mu(s)$  is not available, it should be substituted by a  $\hat{\mu}$  with an *a priori* chosen value. Then the expression for the derivative of  $\lambda$  becomes

$$\dot{\lambda} = \frac{\lambda^2 z}{\mu_r(v - v_{or})} [\mu_r - \hat{\mu}(1 - \sigma) - \frac{1}{T_a} \sigma] \quad (4.21)$$

and substituting in the expression 4.18,

$$\dot{\sigma} = (1 - \sigma)(\mu(s) - \hat{\mu}) - \frac{1}{T_a} \sigma \quad (4.22)$$

so that

$$\sigma \dot{\sigma} = (\sigma - \sigma^2)(\mu(s) - \hat{\mu}) - \frac{1}{T_a} \sigma^2 \quad (4.23)$$

Two elements, namely  $\hat{\mu}$  and  $\frac{1}{T_a}$ , can be set so as to get

$$\sigma \dot{\sigma} \leq 0$$

It should be taken into account that

$$0 \leq \mu(s) \leq \mu_m$$

If  $\hat{\mu}$  is chosen as  $\hat{\mu} = \mu_r$  then

$$\dot{\lambda} = \frac{\lambda^2 z}{\mu_r(v - v_{or})} [\mu_r - \frac{1}{T_a}] \sigma$$

Assuming that

$$\mu_r - \frac{1}{T_a} \leq 0$$

and since

$$\frac{\lambda^2 z}{\mu_r(v - v_{or})} > 0$$

then

$$\begin{aligned} \sigma > 0 &\mapsto \dot{\lambda} < 0 \\ \sigma < 0 &\mapsto \dot{\lambda} > 0 \\ \sigma = 0 &\mapsto \dot{\lambda} = 0 \end{aligned}$$

Moreover,

$$\sigma \dot{\sigma} = (\sigma - \sigma^2)(\mu(s) - \mu_r) - \frac{1}{T_a} \sigma^2$$

with

$$|\mu(s) - \mu_r| < \mu_m$$

Now, only the term  $\frac{1}{T_a}$  is to be set so as to get  $\sigma \dot{\sigma} \leq 0$ . In the worst case,

$$(\sigma - \sigma^2)(\mu(s) - \mu_r) > 0$$

Taking into account that

$$|(\sigma - \sigma^2)(\mu(s) - \mu_r)| < |\sigma - \sigma^2| \mu_m$$

and that for

$$\begin{aligned} |\sigma| > 1, & \text{ the dominant term in } (\sigma - \sigma^2) \text{ is } \sigma^2 \\ |\sigma| < 1, & \text{ the dominant term in } (\sigma - \sigma^2) \text{ is } \sigma \end{aligned}$$

Hence, the following choices may be made

$$|\sigma| > 1, \quad \text{set } \frac{1}{T_a} = 2\mu_m$$

$$|\sigma| < 1, \quad \text{set } \frac{1}{T_a} = \frac{2\mu_m}{|\sigma|}$$

The resulting controller would be:

$$\dot{\lambda} = \frac{\lambda^2 z}{\mu_r(v - v_{or})} \left[ \mu_r - \frac{1}{T_a} \right] \sigma \quad (4.24)$$

$$\frac{1}{T_a} = \begin{cases} 2\mu_m & \text{for } |\sigma| \geq 1 \\ \frac{2\mu_m}{|\sigma|} & \text{for } |\sigma| < 1 \end{cases}$$

Clearly, practical implementation of this scheme makes necessary to add a *dead zone*  $\delta$ . Then the following gain is suggested inside the dead zone:

$$\frac{1}{T_a} = \frac{2\mu_m}{\delta} \quad \text{for } |\sigma| < \delta < 1 \quad (4.25)$$

which forces the system to get into the dead zone. In fact it can be checked that

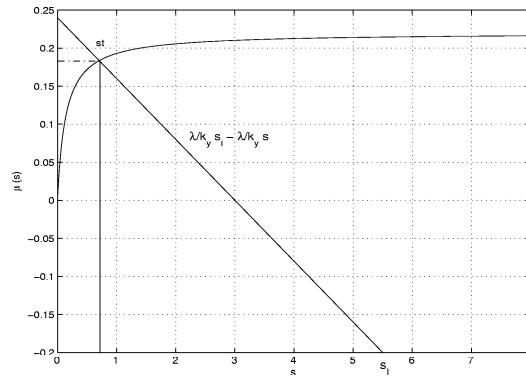
$$\dot{W} < 0 \quad \text{for } \frac{\delta}{2 - \delta} < |\sigma| < \delta$$

From the qualitative analysis in the following subsections, it can be seen that the controller should work always for Monod-like functions but only under certain conditions for Haldane-like ones. In a subsequent section a formal stability proof is presented for the Monod-like case and the Haldane-like case with an adequate saturation on the  $\lambda$ -parameter. For this proof it is convenient to think of the previously presented controller in the framework of sliding mode control. Assuming infinite switching frequency were possible, the controller would lead the system to and make sure that  $\sigma \equiv 0$ . Now, it must be demonstrated that system trajectories on this sliding surface converge to the goal manifold  $Z^*$ . But as it has already been announced, before proceeding with this proof, a qualitative analysis of the controller performance is carried out.

#### 4.4.2 Analysis for monotonous kinetic functions.

In this subsection a qualitative analysis of the closed loop behaviour, or rather of the interaction between plant and controller, for the case of Monod-like kinetic functions is offered.

Figure 4.2 depicts the intersection between a monotonous Monod kinetic function  $\mu(s)$  and the line obtained from  $\dot{s} = 0$  in 4.1 (or 4.4). This point  $s^*$  will correspond to the stationary value of substrate if  $\lambda$  is kept constant. Correspondingly, the broth will grow with specific growth rate  $\mu(s^*)$ . As it is clearly shown, a variation in  $\lambda$  produces a variation in the equilibrium value



**Fig. 4.2.** Equilibrium substrate concentration for Monod kinetics.

$s^*$  of the same sign. Consequently, the growth rate increases for increasing  $\lambda$ , and viceversa.

Now recall the adaptation law 4.24 and notice the design is such that  $(\mu_r - 1/T_a) < 0$  always. The system evolves in the following way:

- When  $\sigma > 0$ ,  $\lambda$  decreases. Therefore:
  - If  $\mu(s) > \mu_r$  the difference will become smaller, as  $\mu(s)$  decreases.
  - If  $\mu(s) \leq \mu_r$  the difference becomes more negative. This will eventually make  $\sigma$  become negative.
- When  $\sigma < 0$ ,  $\lambda$  increases. Therefore:
  - If  $\mu(s) > \mu_r$  the difference will become greater, as  $\mu(s)$  increases. This will eventually make  $\sigma$  become positive, so the first case above will become applicable.
  - If  $\mu(s) \leq \mu_r$  the difference becomes smaller.

#### 4.4.3 Analysis for non-monotonous kinetic functions.

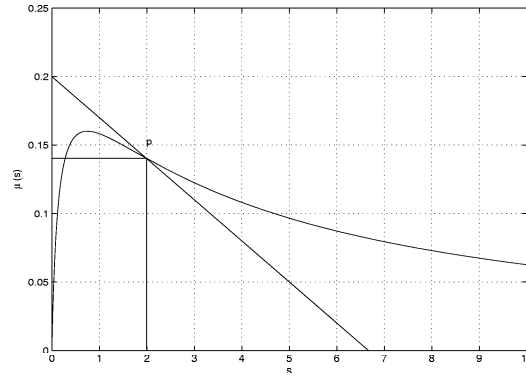
In this subsection a qualitative analysis of the closed loop behaviour, or rather of the interaction between plant and controller, for the case of Haldane-like kinetic functions is offered.

Figure 4.3 depicts the situation for a typical non-monotonous kinetic function.

The main point to consider in this case is the change of sign in the relationship between  $\lambda$  and  $\mu(s)$  when passing over the peak of the kinetic function.

In this case, denoting  $s_{\max}$  the substrate concentration for which the kinetic function  $\mu(s)$  attains its maximum value  $\mu_{\max}$ , the system evolves in the following way:

- When  $\sigma > 0$ ,  $\lambda$  decreases. Therefore:



**Fig. 4.3.** Equilibrium substrate concentration for Haldane kinetics.

- If  $s \leq s_{\max}$ :
  - If  $\mu(s) > \mu_r$  the difference will become smaller, as  $\mu(s)$  decreases.
  - If  $\mu(s) \leq \mu_r$  the difference becomes more negative. This will eventually make  $\sigma$  become negative.
- If  $s > s_{\max}$  (decreasing  $\lambda$  increases  $\mu(s)$ ):
  - If  $\mu(s) > \mu_r$ , as  $\mu(s)$  increases the system moves towards the region  $s \leq s_{\max}$ .
  - If  $\mu(s) \leq \mu_r$  the difference becomes smaller. This will eventually make  $\mu(s_{r,2}) = \mu_r$  at  $s_{r,2}$  in the region  $s > s_{\max}$ . This corresponds to an unstable point. Any small perturbation towards the left will move the system towards the region  $s \leq s_{\max}$ .
- When  $\sigma < 0$ ,  $\lambda$  increases. Therefore:
  - If  $s \leq s_{\max}$ . We have a behaviour like in the monotonous case.
  - If  $s > s_{\max}$  (increasing  $\lambda$  decreases  $\mu(s)$ ).
    - $\mu(s) > \mu_r$ ,  $\mu(s)$  decreases, but moving rightwards. Nevertheless, the system moves so that  $\sigma$  increases, eventually becoming positive and entering in the cases above.
    - $\mu(s) < \mu_r$ ,  $\mu(s)$  decreases and  $\sigma$  keeps negative.

Therefore, in spite of the change of sign in the process gain, the only critical point is the one given by the second intersection of  $\mu_r$  with  $\mu(s)$ , that is,  $s_{r,2}$ . As far as the initial conditions are to the left of this point the proposed robust-adaptive control drives the system towards  $\mu(s) = \mu_r$  at  $s_{r,1}$ .

#### 4.4.4 Stability proof

It has already been shown that state trajectories converge to (the close vicinity of)  $\sigma \equiv 0$ . This section is devoted to demonstrate that system trajectories on this sliding surface asymptotically converge to the goal manifold  $Z^*$ . In other words, we will demonstrate that if the first off-the-manifold error ( $\varphi_1$ )

is maintained at zero, the second off-the-manifold error ( $\varphi_2$ ) also tends to zero (i.e.  $s \rightarrow s_r$ ) and the feedback gain tends to its nominal value  $\lambda_r$  given by (3.4). In the case of non-monotonous kinetics some precautions must be taken.

On the sliding manifold  $\sigma \equiv 0$ , the closed-loop system dynamics can be rewritten as follows:

$$\Sigma_\sigma = \begin{cases} \dot{s} = [-y_s \mu(s) + \lambda(s_i - s)] x \\ \dot{\lambda} = \left[ -\lambda^2 \frac{\mu(s) - \mu_r}{\mu_r} \frac{v}{v - v_{r,0}} \right] x \\ \dot{v} = [\lambda v] x \end{cases} \quad (4.26)$$

where the equation for the evolution of  $\lambda$  has been obtained from equation 4.18 and the sliding mode existence condition ( $\sigma = 0, \dot{\sigma} = 0$ ). Besides, the equation for biomass concentration has been omitted to avoid redundancy. In fact, on the sliding manifold  $\sigma \equiv 0$ ,  $x$  is algebraically dependent on  $\{\lambda, v\}$ :  $x = \frac{z_{r,0}}{v} + \frac{\mu_r}{\lambda v}(v - v_{r,0}) > 0$ . Note also that biomass concentration is bounded since  $v$  only can increase.

On the previously defined system it is possible to apply Theorem 1 in chapter 3 on partial stability. Before defining the necessary Lyapunov and  $\alpha$  functions some definitions are needed:

*Definition:* Let  $\psi(v) = \frac{v}{v - v_{r,0}} : (v_{r,0}, \infty) \mapsto (\infty, 1)$ . See that  $v = v_{r,0} \frac{\psi}{\psi - 1}$  and that the Frechet derivative  $\psi' = -(\psi - 1)^2 / v_{r,0}$ . It must be taken into account that  $v_{r,0}$  is a constant entering in the definition of the reference manifold. The initial condition for volume is  $v_0$  and it is assumed that  $v_0 > v_{r,0}$ . So, actually function  $\psi$  could be defined as a (bounded) mapping from  $(v_0, \infty)$  into  $(\psi_0, 1)$ . A fact that is used in the complementary proof of asymptotic stability below.

*Definition:* Let  $\zeta$  the partial state  $\zeta = \text{col}(s, \lambda)$  and  $\zeta_r = \text{col}(s_r, \lambda_r)$ . Recall that  $s \in \mathcal{S} = (0, s_i)$  and  $\lambda \in \mathfrak{R}^+$ . Let  $\mathcal{M} = \mathcal{S} \times \mathfrak{R}^+$ , and  $M_\sigma$  the region of  $\sigma \equiv 0$  such that  $\zeta \in \mathcal{M}$ .

See that, replacing  $x$  in the last equation of (4.26), yields  $\dot{v} = \mu_r(v - v_{r,0}) + \lambda z_{r,0}$ , which confirms that, on  $\sigma \equiv 0$ , the volume diverges exponentially. As a result,  $\psi \xrightarrow{t \rightarrow \infty} 1$ .

On the other hand, for the partial system  $\Sigma_\sigma$ , define the candidate partial Lyapunov function:

$$V(\zeta, \psi(v)) = \psi \int_{s_r}^s \frac{\mu(\varsigma) - \mu_r}{\mu_r} d\varsigma + (s_i - s_r) \left[ \ln \frac{\lambda}{\lambda_r} + \frac{(\lambda_r - \lambda)}{\lambda} \right]. \quad (4.27)$$

Its time derivative is

$$\begin{aligned} \dot{V}(\zeta, \psi(v)) = & -\psi x \left[ (\psi - 1)\lambda \int_{s_r}^s \frac{\mu(\zeta) - \mu_r}{\mu_r} d\zeta + \right. \\ & \left. \frac{y_s}{\mu_r}(\mu(s) - \mu_r)^2 + \frac{\lambda}{\mu_r}(\mu(s) - \mu_r)(s - s_r) \right]. \end{aligned} \quad (4.28)$$

A function  $\alpha$  such that  $\alpha(\|\zeta\|) \leq V(\zeta, \psi(v))$  is also needed. The following can be used

$$\begin{aligned} \alpha(\zeta) = & \int_{s_r}^s \frac{\mu(\zeta) - \mu_r}{\mu_r} d\zeta + \\ & +(s_i - s_r) \left[ \ln \frac{\lambda}{\lambda_r} + \frac{(\lambda_r - \lambda)}{\lambda} \right]. \end{aligned} \quad (4.29)$$

At this point, it would be interesting to use a result of the Barbashin-Krasovski (or LaSalle) type in order to prove not just stability but asymptotic stability. Two proper solutions are possible:

- a) Using Theorem 2 in [95].
- b) Using results in [92] Chapter 3 for partial stability in the presence of large initial perturbations.

a) Theorem 2 is stated as follows: “Consider the nonlinear dynamical system given by (3.33 in the thesis) and assume  $D \times R^{n_2}$  is a positive invariant set with respect to (3.33). Furthermore, assume there exist functions  $V : D \times R^{n_2} \mapsto R$ ,  $W, W_1, W_2 : D \mapsto R$  such that  $V(\cdot, \cdot)$  is continuously differentiable,  $W_1(\cdot)$  and  $W_2(\cdot)$  are continuous and positive definite,  $W(\cdot)$  is continuous and nonnegative definite, and, for all  $(x_1, x_2) \in D \times R^{n_2}$ ,

$$W_1(x_1) \leq V(x_1, x_2) \leq W_2(x_1) \quad (4.30)$$

$$\dot{V}(x_1, x_2) \leq -W(x_1) \quad (4.31)$$

Then there exists  $D_0 \subseteq D$  such that for all  $(x_{10}, x_{20}) \in D_0 \times R^{n_2}$ ,  $x_1(t) \mapsto \mathcal{R} \triangleq \{x_1 \in D : W(x_1) = 0\}$  as  $t \mapsto \infty$ . If, in addition,  $D = R^{n_1}$  and  $W_1(\cdot)$  is radially unbounded, then for all  $(x_{10}, x_{20}) \in R^{n_1} \times R^{n_2}$ ,  $x_1(t) \mapsto \mathcal{R} \triangleq \{x_1 \in R^{n_1} : W(x_1) = 0\}$  as  $t \mapsto \infty$ .”

In order to complete the proof of AS, the following remark in the same paper must be taken into account. Remark: “Theorem 2 shows that the partial system trajectories  $x_1(t)$  approach  $\mathcal{R}$  as  $t$  tends to infinity. However, since the positive limit set of the partial trajectory  $x_1(t)$  is a subset of  $\mathcal{R}$ , Theorem 2 is a much weaker result than the standard invariance principle wherein one could conclude that the partial trajectory  $x_1(t)$  approaches the largest invariant set  $\mathcal{M}$  in  $\mathcal{R}$ . This is not generally true for partially stable systems since the positive limit set of a partial trajectory  $x_1(t), t \geq 0$  is not an invariant set. However, in the case where  $f_1(\cdot, x_2)$  is periodic, almost periodic or asymptotically independent of  $x_2$ , then an invariance principle for partially stable systems can be derived.”

Taking the function  $V$  already defined in the existing proof of stability, it is possible to define positive definite functions  $\bar{V}(\zeta) \triangleq V(\zeta, \psi_0)$  and  $\underline{V}(\zeta) \triangleq V(\zeta, 1)$  such that

$$\underline{V}(\zeta) \leq V(\zeta, \psi) \leq \bar{V}(\zeta) \quad (4.32)$$

An additional nonnegative definite function can be defined as  $W(\zeta) \triangleq -\dot{V}(\zeta, 1)$  so that

$$\dot{V}(\zeta, \psi) \leq -W(\zeta) \quad (4.33)$$

Finally, the equations for the evolution of  $s$  and  $\lambda$  on the sliding manifold  $\sigma \equiv 0$  are asymptotically independent of  $v$ . That is, as  $v$  diverges,  $\psi \mapsto 1$  and  $x \mapsto \mu_r/\lambda$ , thus approaching to the system

$$\begin{aligned} \dot{s} &= (-y_s \mu(s) + \lambda(s_i - s)) \frac{\mu_r}{\lambda} \\ \dot{\lambda} &= -\lambda(\mu(s) - \mu_r) \end{aligned} \quad (4.34)$$

Consequently,  $\zeta_r$  is a globally asymptotically stable partial equilibrium point for the partial system  $\Sigma_\sigma$ .

b) Another line could have been followed using results in [92]. This reference deals mainly with systems  $\dot{x} = X(t, x) \quad X(t, 0) = 0$  with  $x^T = (y, z)$  for which stability is sought with respect to the  $y$ -variables. (See also the thesis document pages 35-36). According to [92] Chapter 0, theorems of the Barbashin-Krasovski can be applied in the autonomous case provided the  $z$ -variables are bounded. In Chapter 3 a theorem giving conditions for AS is given for the case in which  $z_0$  may be large. For this to be applicable to our case, notice it is possible to use function  $\psi$  directly in the equations for the evolution of  $s$  and  $\lambda$ . Also, using this new variable, the equation for volume transforms into

$$\dot{\psi} = -(\psi - 1)\psi\lambda x \quad (4.35)$$

The actual application of these theorems is left as a future task.

For non-monotonous kinetic functions, e.g. Haldane, the previous results about stability are only local. Actually, the system may present two equilibrium points. Let denote  $s_m$  the substrate concentration at which the growth rate is maximum,  $s_r < s_m$  and  $s^r > s_m$  the substrate concentrations satisfying  $\mu(s_r) = \mu(s^r) = \mu_r$ . Locally around  $s_r$ , the kinetic function behaves as a monotonous function. Then,  $V(\zeta, \psi(v))$  is locally positive definite around  $\zeta_r$ , whereas  $\dot{V}(\zeta, \psi(v))$  is locally negative semi-definite and  $\zeta_r$  is the largest invariant set for which  $\dot{V} = 0$ . Then,  $\zeta_r$  is a locally asymptotically stable equilibrium point for the partial system  $\Sigma_\sigma$ , and the original system on  $\sigma \equiv 0$  locally asymptotically converges to the goal manifold  $Z_{r,0}$ .

*Definition:* Let  $\mathcal{S}^r = \{s \in \mathcal{S} \mid s < s^r\}$ ,  $\mathcal{L}^r = \left\{ \lambda \in \mathbb{R}^+ \mid \lambda < \lambda^r = \frac{y_s \mu_r}{s_i - s^r} \right\}$ ,  $\mathcal{M}^r = \mathcal{S}^r \times \mathcal{L}^r$  and  $M_\sigma^r$  the region of  $\sigma \equiv 0$  where  $\zeta \in \mathcal{M}^r$ .



It is clear from the previous expressions that  $M_\sigma^r$  is a domain of attraction of  $\zeta_r$  on the sliding manifold  $\sigma \equiv 0$ , that is a region of convergence towards  $Z_{r,0}$  on  $\sigma \equiv 0$ . Nevertheless, if the substrate concentration is initially very high, the system state might reach (the close vicinity of) the sliding manifold outside the domain of attraction, leading to undesired unstable dynamics.

A possible solution suggested here is to modify the adaptation law so that the trajectories are steered to reach the attractive region  $M_\sigma^r$  of the sliding manifold  $\sigma \equiv 0$ . A natural way of avoiding the aforementioned undesired dynamics is limiting the feeding governed by  $\lambda$  and  $s_i$ . See chapter 3 on the invariant control. According to this, the adaptation law is modified by incorporating the saturation function

$$g(w_1, w_2) = \begin{cases} 0 & \text{if } w_1 \geq 0 \wedge w_2 \geq 0 \\ 1 & \text{otherwise.} \end{cases} \quad (4.36)$$

where  $w_1 = \lambda - \bar{\lambda}$  and  $w_2 = -\sigma$ . To complete the analysis, we need to show that despite saturation, all state trajectories finally reach the vicinity of  $\sigma \equiv 0$ . In fact, during saturation, the derivative of the function

$$W = \frac{1}{2}\sigma^2$$

becomes

$$\dot{W} = -\mu\sigma^2 + (\mu - \mu_r)\sigma. \quad (4.37)$$

On one hand, if  $\sigma > 0$ , saturation becomes inactive ( $g(\cdot, \cdot) = 1$ ) and  $\dot{\lambda}$  is negative. Thus, inequality  $\dot{W} < 0$  holds, i.e. the trajectory points towards  $\sigma \equiv 0$ . On the other hand, if  $\sigma < 0$ ,  $\lambda$  remains at its limit value. Therefore, to approach  $\sigma \equiv 0$ , (4.37) should be negative whenever  $\sigma < 0$ . It can be shown that this is true, possibly except for an initial period of time. In fact, as  $\lambda$  is maintained fixed at  $\bar{\lambda}$ , the partial state  $\zeta$  will finally reach  $\mathcal{M}^r$ , and moreover, will converge to  $\bar{\zeta} = (\bar{s}, \bar{\lambda}) \in \mathcal{M}^r$ , where  $\bar{s}$  is the substrate concentration at which the solid line crosses the kinetic function. Since  $\mu(\bar{s}) > \mu_r$ ,  $\dot{W}$  will, sooner or later, become negative. Consequently, trajectories will finally point towards  $\sigma \equiv 0$  from both sides as desired.

*Observation :* Note that although it is not necessary to assure convergence toward  $Z_{r,0}$ , limiting  $\lambda$  may also be used in the case of Monod-like kinetic functions to improve the transient from certain initial conditions. Note that a kind of windup effect may appear due to the saturation of Monod functions and the integrator implicit in the control law. Effectively, in order to reach and maintain the process state on the sliding manifold  $\sigma \equiv 0$ , a large overshoot in  $\lambda$  may appear, leading to an excess of feeding and a large settling time. Limiting appropriately the value of  $\lambda$ , the substrate concentration

is bounded hence avoiding strong saturation of the growth rate and the associated windup effect. Based on the previous analysis, convergence to the equilibrium point on the sliding manifold  $\sigma \equiv 0$  is still guaranteed despite  $\lambda$  limitation provided  $\bar{\lambda} > \lambda_r$ .

## 4.5 Conclusions

In this chapter, three different designs have been suggested to cope with uncertainty. All of them are composed of two parts: the invariant control plus a correction or adaption on  $\lambda$  (and in the first case also on  $s_i$ ). The results on the invariant control can also be used to extend their validity to the case in which the kinetic functions are Haldane-like. The first design is relatively simple but requires more actuators. Since one of the goals was to use a minimum of resources other schemes were sought after. The second design was a first attempt in this direction and served as a preparation for the robust adaptive controller. This one, the most sophisticated in its conception, has given good experimental results as shown in the following chapter.

## 5 Practical results

### 5.1 Introduction

In this chapter the practical behaviour of the controllers presented before is shown using simulations, and in the last two cases, experimental results are also included. These were obtained in fermentations with a local strain of *S. cerevisiae* the T73(CECT 1894). All experiments were performed in a 5 liters B. Braun Biotech bioreactor, model Biostat B.

### 5.2 Results. Flatness

In this subsection some results corresponding to the control using two PIs are presented. There is no formal analysis of robustness, leaving it as a future goal. The parameters with respect to which the behaviour in closed loop is most sensitive have been determined and afterwards a series of simulations have been carried out. Parameter values from real processes have been used so as to have an idea of the behaviour it might be expected in a real application.

In the following pages, the simulations using the complete control scheme are shown. In them, besides considering the uncertainty in the initial conditions, the value of the yield coefficient  $y_s$  is varied. This coefficient is the most important for the system behaviour. The experimental values for the plant parameters and the control are shown in tables 5.1 and 5.2. These experimental values correspond with those obtained for a local strain of *Sacharomices Cerevisiae*, the T73.

**Table 5.1.** Plant parameters and nominal exosystem.

plant	
$\mu_m$	0.22
$y_s$	1.43
$k_s$	0.14
exosystem	
$\mu_r$	0.11

**Table 5.2.** Controller parameters.

control			
$\lambda_n$	0.0079	$s_{in}$	20
$k_{p1}$	0.01	$k_{p2}$	10
$T_{I1}$	100	$T_{I2}$	100

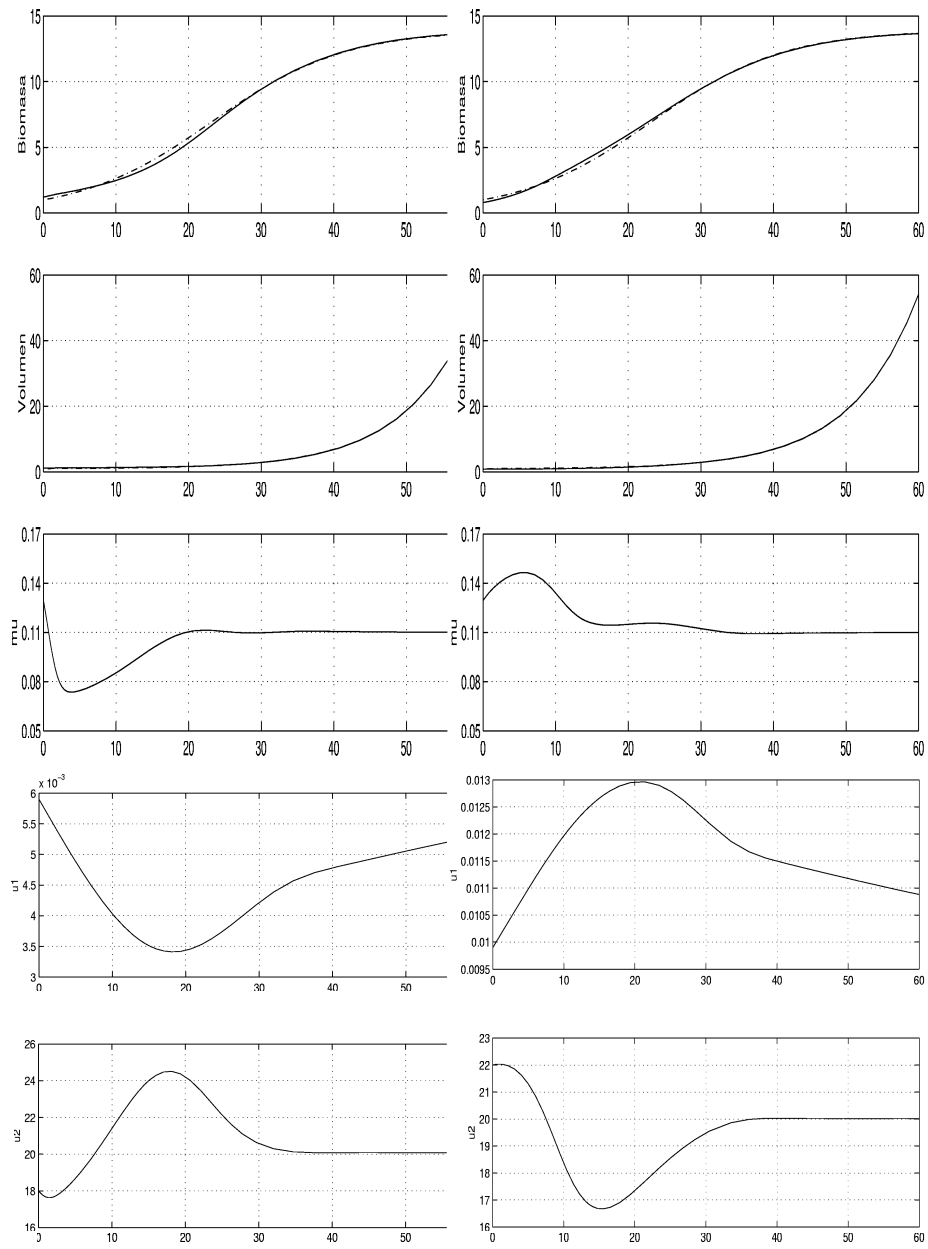
Errors in the initial conditions have been considered and  $y_s$  takes the values 1.2, 1.43, 1.66 which are over and below the nominal value.

Table 5.3 shows the correspondence between the graphics and the different combinations of initial conditions and values of the yield coefficient.

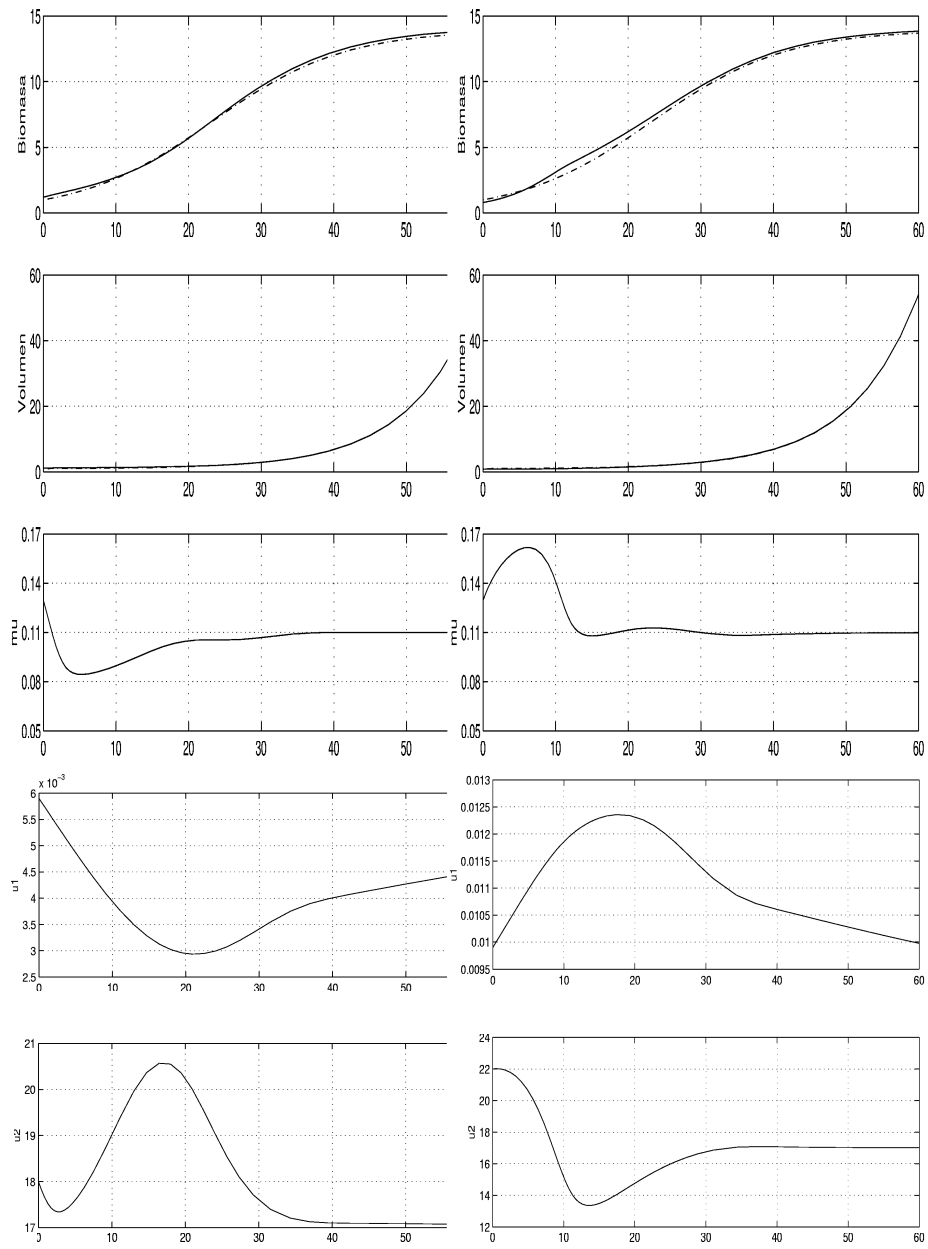
**Table 5.3.** Table of results

$e$ vs. $y_s$	1.2	1.43	1.66
$e_x, e_v < 0$	fig. 3	fig. 1	fig. 5
$e_x, e_v > 0$	fig. 4	fig. 2	fig. 6

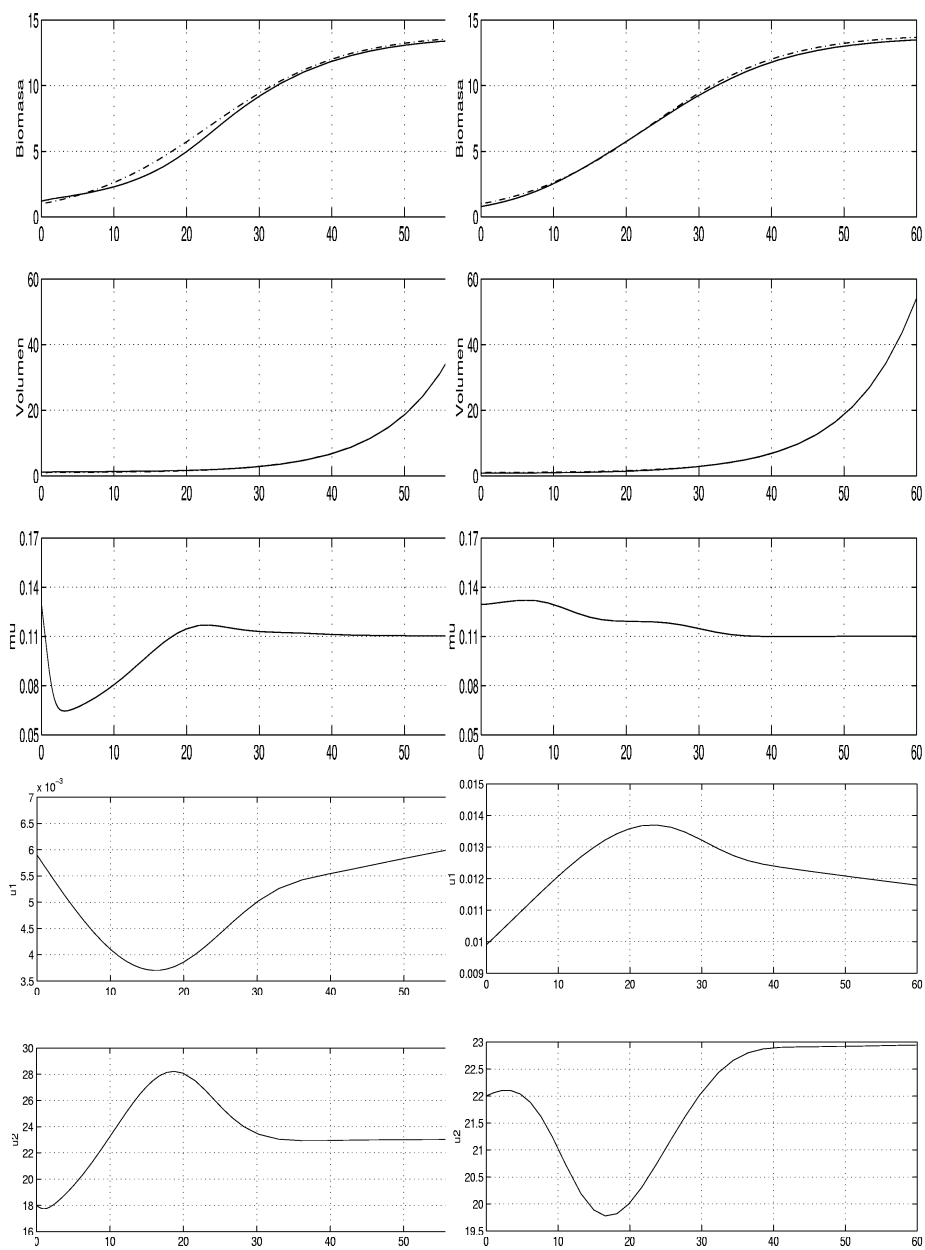
The results show control is very robust against uncertainty both in the model and in the initial conditions, although transients tend to be longer when  $e_x, e_v > 0$  and  $y_s > y_{nominal} = 1.43$ . On the other hand, control goals are achieved quickly, the error during the transient being very small. Besides the regulation of the specific growth rate, a very important task from the physiological point of view, final biomass concentration and volume are very close to those of the exosystem. See the first two graphs. Thus, it is possible to reach an adequate quantity of biomass.



**Fig. 5.1.** LEFT-Nominal system and exosystem. Negative error in the initial conditions. RIGHT-Nominal system and exosystem. Positive error in the initial conditions.



**Fig. 5.2.**  
 LEFT-System and exosystem.  $y_s = 1.2, (e_x, e_v) < 0$ .  
 RIGHT-System and exosystem.  $y_s = 1.2, (e_x, e_v) > 0$ .



**Fig. 5.3.**  
 System and exosystem.  $y_s = 1.66, (e_x, e_v) < 0$ .  
 System and exosystem.  $y_s = 1.66, (e_x, e_v) > 0$ .

### 5.3 Results. Proportional.

The results in the corresponding section of chapter 4 were checked on a series of simulations, prior to its application on real fermentations. The simulated responses showed that:

- Supposing perfect model and error in the initial conditions (i.e. initial conditions not in the manifold to track), the best control was  $u = (\lambda + k_p \xi_1)z$  with  $k_p = 0$ . The invariant control alone.
- Supposing modeling error (specifically in the yield parameter  $y_s$ , which is the critical one) some correction of the nominal invariant control action is necessary.

The results corresponding to a real experiment are shown below. A fermentation with a genetically modified strain T73 of yeast *S. cerevisiae* was carried out. In particular, at the IATA the recombinant yeast *Saccharomyces cerevisiae*  $T_{73}$  with the plasmid YepCR21 was studied with the purpose of finding a feeding protocol to optimize the expression of different heterologous enzymes. An exceptional modification of the limit between oxidative and oxidoreductive behaviour was found. So, this strain did only present oxidoreductive behaviour. This result led to the investigation of the use of ethanol as substrate in fed-batch operation. A natural procedure in such a case would be to start with a glucose batch, producing ethanol and an initial amount of biomass. Once the glucose is exhausted, the ethanol is used as carbon source, using in turn batch and fed-batch operation. This procedure has been investigated for the  $T_{73}$  with different plasmids.

In the experiment shown below, the yeast is fed first using batch operation on glucose for 20 hours, as shown in figures 5.4 and 5.5. The control action shown in figure 5.5 is filtered, so the actual flow evolves smoothly. Once the glucose is exhausted, the yeast consumes the ethanol produced in the previous phase, after a short adaptation period. At  $t = 29$  hours the controlled fed-batch is started, feeding with ethanol. In figure 5.6 an instantaneous estimation of the specific growth rate is shown. Its is kept constant for a long period. After 20 hours the behaviour of the microorganism changes with time (the experiment took three days) due to several factors. This change mainly affects the yield coefficient. Since the parameter  $k_p$  was set to a relatively small value, and no integral action was added, the error in steady state is significant. Yet, the controller manages to keep the specific growth rate constant.



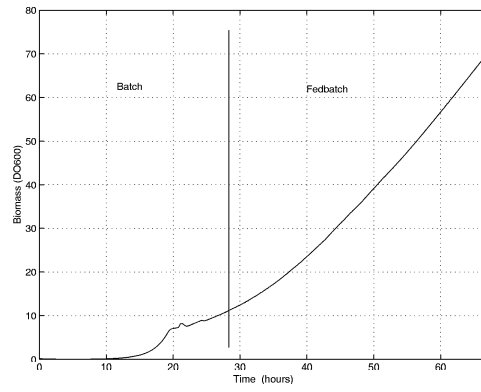


Fig. 5.4. Biomass concentration.

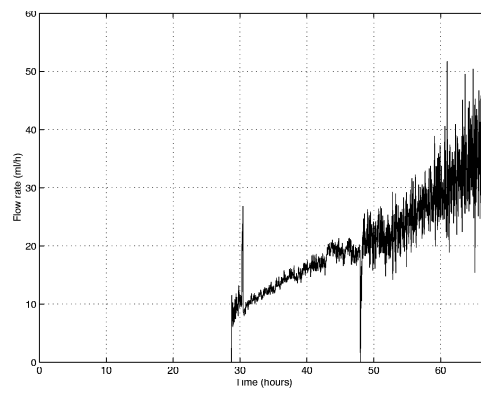


Fig. 5.5. Flow rate.

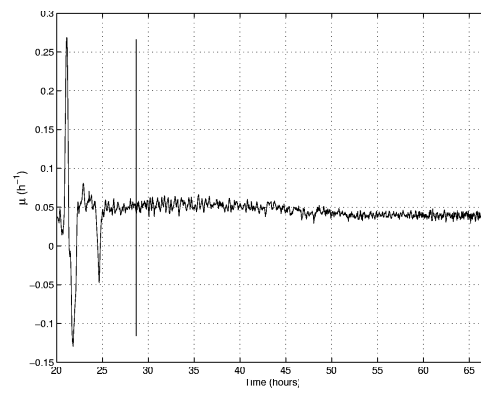


Fig. 5.6. Specific growth rate estimation.

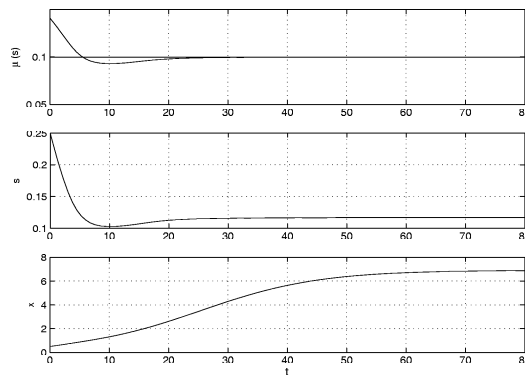
## 5.4 Results. Robust-adaptive controller

In this section, some simulations and experimental results are presented which show the behaviour of the robust adaptive controller. The real experiments were carried out using a non modified or natural *Saccharomyces cerevisiae*  $T_{73}$ . Thus, three fed-batch fermentations on glucose are included. In all of them the goal was to keep a relatively low specific growth rate so as to avoid the formation and accumulation of ethanol.

### 5.4.1 Simulations

Before proceeding with the experiments, some simulations are provided showing the system behaviour both under ideal conditions and in the presence of noise, measuring errors, model errors, etc.

- System with Monod-like kinetic functions. Conditions are assumed to be ideal except for figure 5.11 in which simulations are compared for the nominal system and systems with errors in the yield coefficient  $y_s$ , the  $K_s$  and a maintenance constant . Figures 5.7 and 5.8 show the results when the initial concentration of substrate is slightly over the required one, which would be the typical case when a previous batch has been performed to launch the inoculus. The value of  $x_{r,0}$  is over the real one. As shown, the specific growth rate very quickly enters a small vicinity of the desired one. Figures 5.9 and 5.10 show the results for the case when the initial concentration of substrate is much higher than the required for keeping the desired specific growth rate ( $\mu_r = 0.1$ ). It is important to keep in mind that only an upper bound on the maximum growth rate is required.



**Fig. 5.7.** Time evolution specific growth rate, substrate and of biomass.

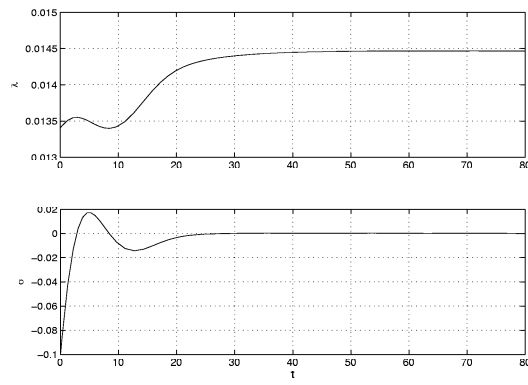


Fig. 5.8. Time evolution of  $\lambda$  and  $\sigma$ .

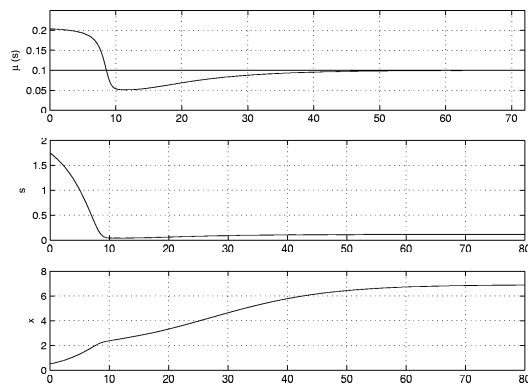


Fig. 5.9. Time evolution of specific growth rate, substrate and biomass.

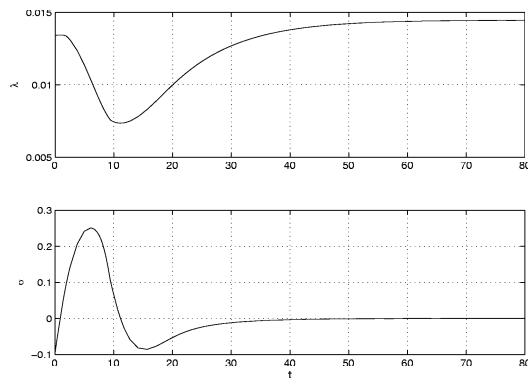
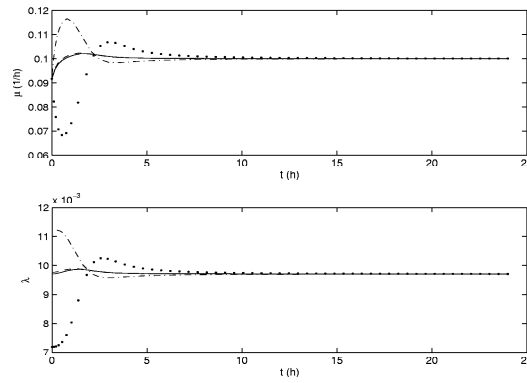
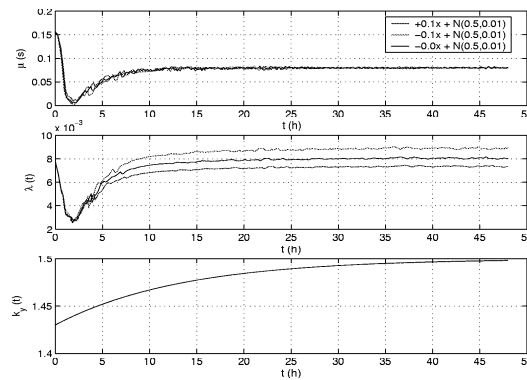


Fig. 5.10. Time evolution of  $\lambda$  and  $\sigma$ .

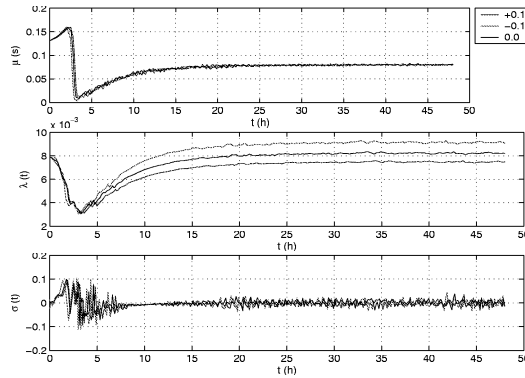


**Fig. 5.11.** Nominal system - ;  $e_{K_s} = +100\%$  - ;  $e_{Y_s} = +20\%$  -.- ;  $e_m = -100\%$  \*\*

- System with Haldane-like kinetic functions. Noise and measuring errors are assumed to be present. The reference is  $\mu_r = 0.08$ . Figure 5.12 shows the results for measuring errors in biomass including a term proportional to biomass concentration itself and gaussian noise. The latter is also considered in the measure for volume. Finally, the yield coefficient  $y$  ( $k_y(t)$  in the graphic) changes with time. Figure 5.13 shows a similar scenario but beginning from a higher glucose concentration in the decreasing side of the haldane function. Note that the adjustable gain  $\lambda$  converges to different steady state values.



**Fig. 5.12.** Time evolution of the specific growth rate  $\mu$ ,  $\lambda$  and the yield coefficient.



**Fig. 5.13.** Time evolution of the specific growth rate  $\mu$ ,  $\lambda$  and the normalized error  $\sigma$ .

### 5.4.2 Experiments

In this subsection, the results mentioned in the introduction of three fed-batch fermentations on glucose are shown. All of them are preceded by a batch which is not included. The product of biomass concentration by volume, i.e. the absolute biomass, is in logarithmic scale and a straight line with the slope corresponding to the reference specific growth rate is added to facilitate comparison.

The results of the first experiment are shown in Fig. 5.14. After an initial batch with a glucose concentration of 5g/L (Fig. 5.14(d)), the controlled fed-batch was switched on at  $t_0 = 7.65\text{h}$ , when the glucose in the medium was almost exhausted. The concentration of glucose in the feeding flow was set to 20g/L.

The constants of the goal manifold were set to  $z_{r,0} = z(t_0)$  and  $v_{r,0} = 0.9v(t_0)$ , whereas the initial value of  $\lambda$  was set at  $\lambda(t_0) = 1.3e - 3L(gh)^{-1}$ . Under these conditions, the initial value of the normalized off-the-manifold error results  $\sigma(t_0) = -2.3$ . Then, the control algorithm increases  $\lambda$  in order to approach the sliding surface  $\sigma \equiv 0$  (Fig. 5.14(c)). The long term variation in  $\lambda$  (Fig. 5.14(c)), which is commonly observed in all long experiments, can be explained as an adaptation to the varying yield coefficient  $y_s$ . For this reason, the control strategies that use a-priori estimation of  $y_s$  usually fail to regulate the specific growth rate during the whole experiment.

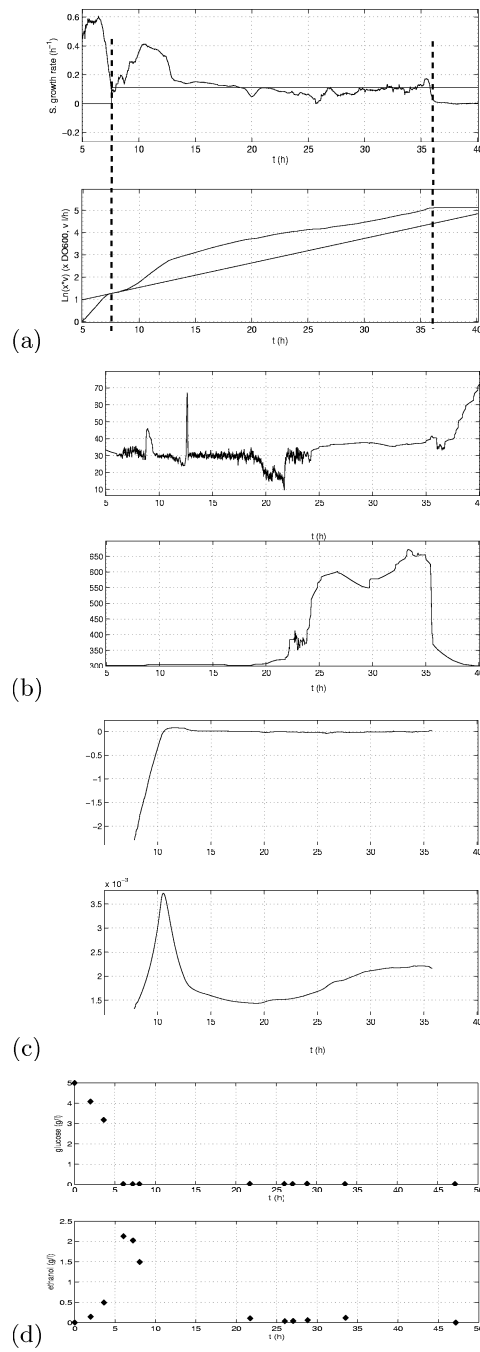
The integral action inherent to the controller causes an initial overshoot in the specific growth rate (Fig. 5.14 (a)) for some 4 hours. This transient overshoot could have been reduced by choosing  $z_{r,0}$ ,  $v_{r,0}$  and  $\lambda(t_0)$  so that  $\sigma(t_0) \cong 0$ . Anyway, the large initial value of  $\sigma(t_0)$  allows us to corroborate the reaching properties towards  $\sigma \equiv 0$  of the algorithm (Fig. 5.14(c)). During the rest of the experiment, the specific growth rate  $\mu$  keeps around the desired value but for some periods of time (around  $t = 20\text{h}$  and  $t = 25\text{h}$ ).

At these periods,  $\mu$  drops due to limitation in the oxygen supply, as seen in figure 5.14(b) looking at the decrease of  $pO_2$  at  $t = 20$ h and the increase of the stirrer speed at  $t = 25$ h. This behaviour occurs because there was a deficient control loop for  $pO_2$  in the experiment, and  $O_2$  was not considered as a limiting substrate in the model. Actually, whenever this limitation appears, one should improve the oxygen transfer rate by means of the air supply and stirrer speed and/or demand for a lower specific growth rate.

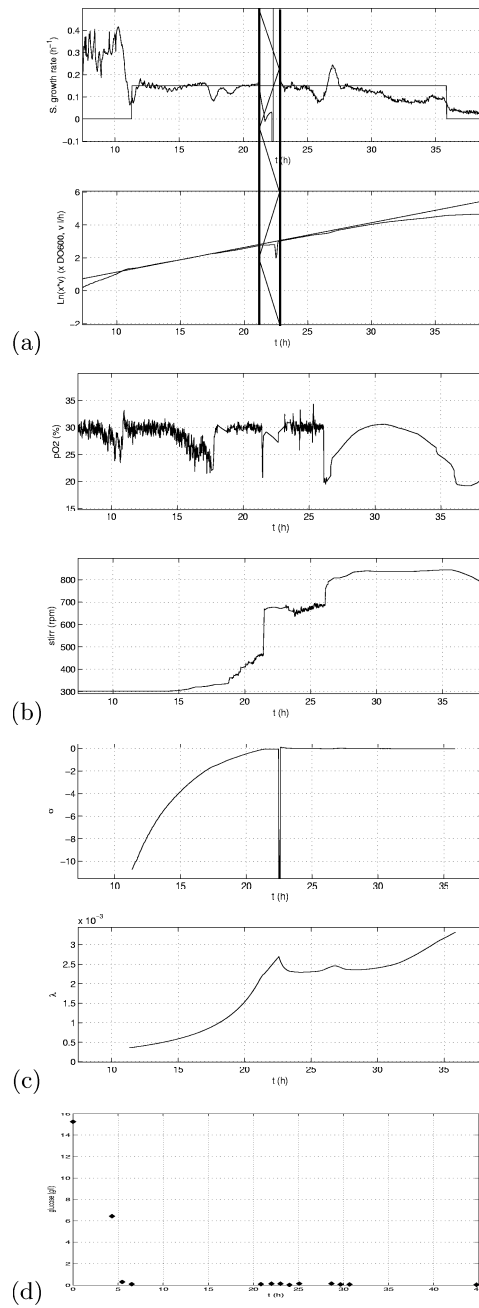
Finally, it is important to stress the low values of glucose in the medium after the initial batch. They kept at values around 0.023g/L throughout the experiment. Their order of magnitude is close to that of measurement noise. Therefore, a control strategy based somehow on measurements or estimation of the substrate is not feasible in practice. As for ethanol, the low specific growth rate permitted to avoid its formation.

The second experiment shows a similar pattern. The specific growth rate is kept at the reference until oxygen supply drops after 18 hours of the experiment. Then, it recovers except for a short period of time spanning the criss-crossed area in which there was a pump failure. From 27 hours onwards, it becomes very difficult to maintain an adequate oxygen level (Fig. 5.15 (b)). Consequently,  $\mu$  slowly decreases and  $\lambda$  increases trying to compensate for the apparent change in the yield coefficient. See figure 5.15(a) and (c). Previously, there is a curious oscillation which does not clearly coincide with an oxygen shortage. This phenomenon was seen again in the last experiment. Finally, the off-line measurements of glucose are shown in fig. 5.15 (d).

In the third experiment, some of the difficulties inherent to these systems are illustrated. From 10 to 20 hours the system would not respond as expected despite the fact oxygen was in adequate supply and  $\lambda$  was increasing quickly. See fig. 5.16. Eventually, at 25 hours, growth simply stopped. After two strong pulses of glucose and fresh medium, the system began to behave following the pattern of the other two experiments. Notice that, in fact, the value of  $\lambda$  is roughly the same after and before the break. In the period between 26 and 42 hours, the specific growth rate  $\mu$  is kept at the reference except for short periods of time. The first ones coincide with the appearance of problems in the oxygen supply. See fig. 5.16(b). But some of these exceptions, particularly at the end, look like sudden oscillations. A possible explanation may be offered in [156]. According to this article, the unpredictable appearance and disappearance of oscillations has been observed in cultures of *S. cerevisiae*. This phenomenon is related to the formation of distinct cell subpopulations via a mechanism known as cell cycle synchrony. In particular, biomass segregates into two big groups. One of mature or *mother* cells and one of *new-born* cells.

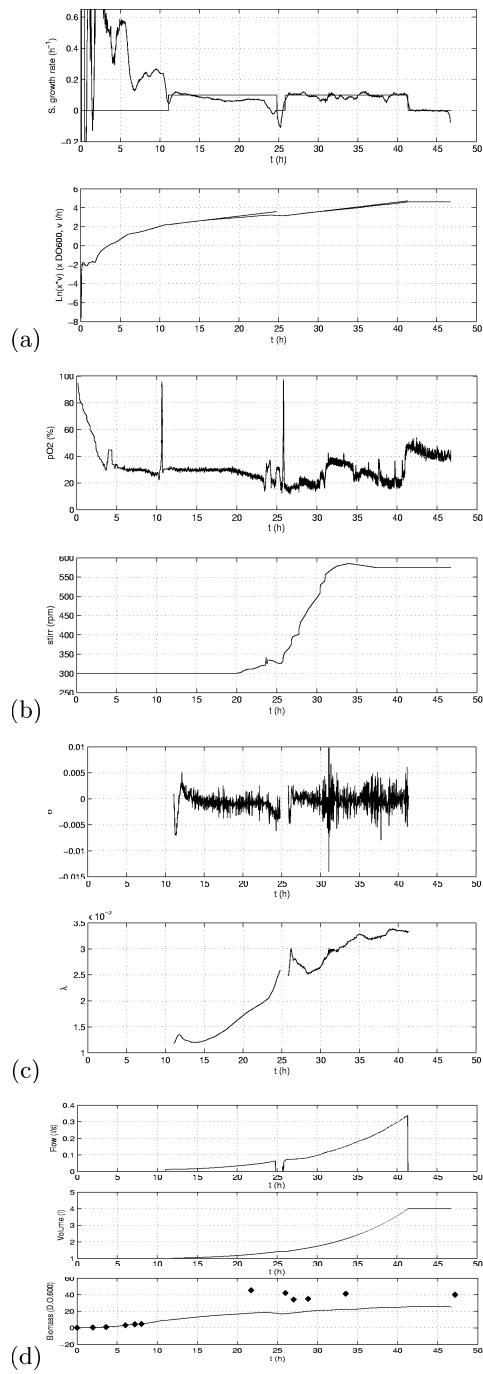


**Fig. 5.14.** Fed-batch on *S. cerevisiae* T<sub>73</sub>. (a) Specific growth rate,  $\ln(z)$  and line with slope  $\mu_r$ . (b)  $pO_2$  (%) and stirrer (r.p.m.). (c)  $\sigma$  and  $\lambda$ . (d) Off-line measurements of glucose and ethanol.



**Fig. 5.15.** Fed-batch on *S. cerevisiae*  $T_{73}$ . (a) Specific growth rate,  $\log(z)$  and line with slope  $\mu_r$ . (b)  $pO_2$  (%) and stirrer (r.p.m.). (c)  $\sigma$  and  $\lambda$ . (d) Off-line measurements of glucose





**Fig. 5.16.** Fed-batch on *S. cerevisiae* T73. (a) Specific growth rate,  $\log(z)$  and line with slope  $\mu_r$ . (b)  $pO_2$  (%) and stirrer (r.p.m.). (c)  $\sigma$  and  $\lambda$ . (d) Flow, volume and biomass



## 6 Conclusion.

Bioreactors are very complex systems with non-linearity, uncertainty, partial equilibria, etc. In addition, as opposed to the case of (electro)-mechanical systems, there are not so many available results. Often the problem is solved for bioreactors in continuous mode or unrealistic hypothesis, such as the ability of measuring the full state on-line, are assumed.

It was decided to approach the general problem beginning with particular cases, and generalize the solutions later on. Therefore, all the work is centred around the basic models described in section 2.3 for pure cultures with only one limiting substrate and assuming oxygen is in excess. Also, it was decided to use a minimum number of measures and actuators. These demands are a consequence of practical considerations, but on the other hand it is also interesting in itself to analyze what can be done with a minimum of information. Other central factors, namely nonlinearity and uncertainty, determine the techniques to be used and the search for a robust/adaptive controller.

### Attainments

Several important goals have been attained:

1. First of all, the determination of a reduced number of *standard* models of bioreactors after an extensive literature search. These models have relatively few state variables and a couple of particular structures represent most applications for pure cultures with one limiting substrate. Moreover, even if multisubstrate and/or multiorganism systems were taken into account it would still be possible to work with relatively simple models with similar structures. Models for mixed cultures have not been dealt with but some examples can be found in [55] and [56].
2. An invariant control, which can be seen as a closed loop analogue of the usual exponential feeding law, has been suggested. It provides the basis for subsequent designs. In particular, it is a part of the controllers presented in chapter 4 and the geometric approach in analyzing this law has supplied new coordinates useful in defining an *off-the-manifold* error variable. Besides, a local stability proof was obtained that encouraged the search for a global one. This is provided in the final section of chapter

- 3 and enabled the treatment of those problems in which Haldane-like kinetic functions are present.
3. Three designs for the regulation of the specific growth rate are suggested. The first one is based on flatness and uses two PIs. The second one is based on the geometric control techniques developed by Fradkov et al. It has been shown that the problem can be cast as one of partial stability, and the corresponding techniques have been used to analyze it. Using many elements and ideas of this design, a robust adaptive controller has been developed with good experimental results.
  4. The functionality of the previously cited controllers has been extended to the case of non-monotonous (Haldane-like) kinetic functions using the above mentioned results on the invariant control.
  5. Several experiments have been carried out using a strain of the *S. cerevisiae*. The results corresponding to the second result are good, in the sense that a constant specific growth rate is kept constant, but a steady state error appeared. This is solved with the third design which adapts the parameter  $\lambda$  as required by the process, which not only is uncertainly known but also changes with time.

### Future lines

Possible future lines are enumerated in the following:

- A complete and rigorous analysis of systems with inhibitor product. Here, some guidelines are given on how the elements developed in the thesis could be combined to give an almost complete solution to the control of fed-batch bioreactors in one of the standard forms of chapter 2. Let us begin with the more difficult case

$$\begin{aligned}
 \dot{x} &= \mu(s, p)x - Dx \\
 \dot{s} &= -y_{sx}\mu(s, p)x - y_{sp}\pi(s, p)x + D(s_i - s) \\
 \dot{p} &= \pi(s, p)x - Dp \\
 \dot{v} &= Dv
 \end{aligned} \tag{6.1}$$

If it were possible to regulate the product concentration  $p$  at a given value  $p_r$  then

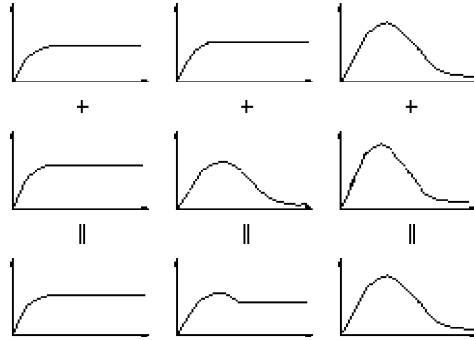
$$\begin{aligned}
 \dot{x} &= \mu(s, p_r)x - Dx \\
 \dot{s} &= -y_{sx}\mu(s, p_r)x - y_{sp}\pi(s, p_r)x + D(s_i - s) \\
 \dot{v} &= Dv
 \end{aligned} \tag{6.2}$$

This could be achieved easily if  $p$  were measured on-line, using the invariant control suggested in chapter 3 and a simple PI for the correction of the

factor  $\alpha$  mentioned there. If no measure of  $p$  is available the problem is much more complex and still open. Going back to system 6.2, it can be checked that the equation for substrate is equivalent to

$$\dot{s} = -\sigma(s)x + D(s_i - s) \quad (6.3)$$

where most often one of the situations described in figure 6 will be found.



**Fig. 6.1.** Superposition of kinetic functions.  $\sigma = \mu + \pi$ .

In principle, the results on the invariant control allow us to work only with the increasing part of both  $\mu$  and  $\sigma$ , so the situation is essentially similar to that studied in the thesis.

- The study of multi-substrate systems, particularly those in which oxygen is the second limiting substrate. Systems with one substrate and one inducer are also very interesting.
- Of importance for the previous goals and in itself, would be to find a stability proof of the invariant control for the case with product. Showing, clearly, the possible extra conditions on the kinetic functions or certain parameters such as  $s_i$  necessary for global stability.

Finding the corresponding invariant control for multi-substrate models would also be important as a first step in generalizing the control structures developed in the thesis.

Finally the already obtained results could be used for analyzing existing controllers as suggested at the end of chapter 3.

- As for the robust adaptive controller, it has been checked in simulations and in a first qualitative analysis that a high gain is not necessary and that a continuous controller with a constant finite gain such as  $1/T_a = 2 * \mu_m$  for all  $\sigma$  could be used with the same qualitative results. The search for a stability proof remains an open problem.

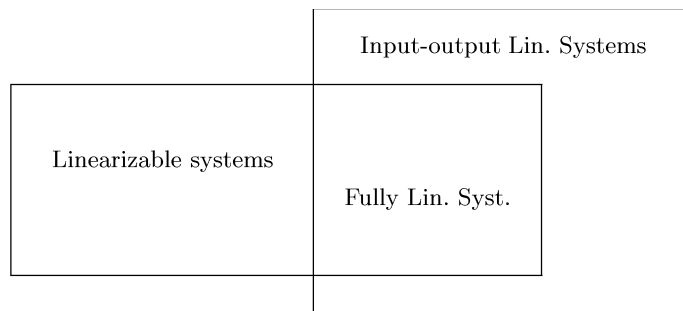
- Finally, it would be interesting to analyze how to take advantage of possibly available extra information such as an estimation of the specific growth rate even if it is not very precise.

# A Flatness

## A.1 Introduction.

In this appendix the concept of flatness, its relation to exact linearization or more specifically exact linearization of state space equations, and a first analysis of its applicability to bioreactors, are explained.

Whenever a system has the latter property mentioned above is called linearizable (See figure A.1).

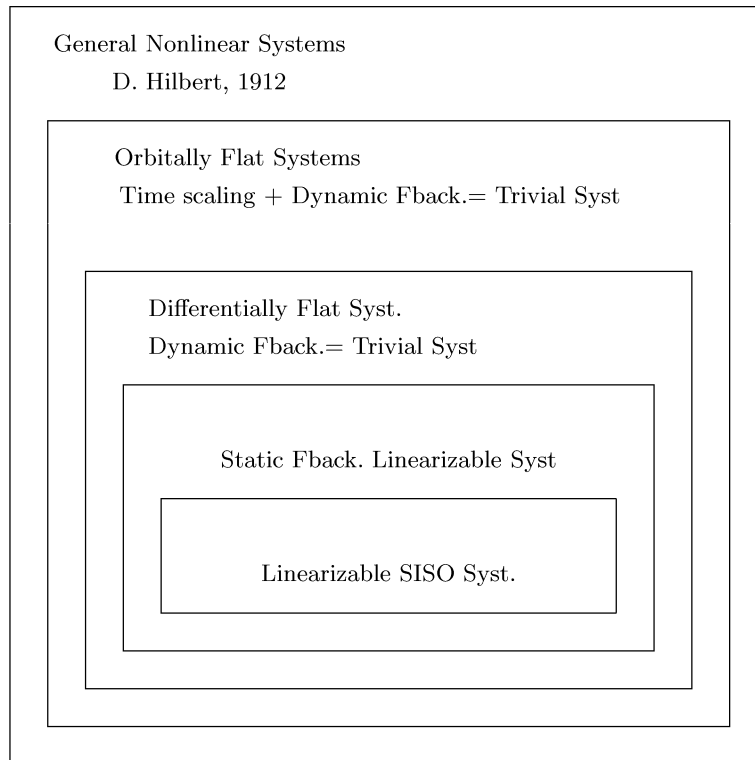


**Fig. A.1.** Exact Linearization

More specifically, for a linearizable system it is possible to find some (dummy) outputs such that the state space equations can be put in linear form through a (dynamic) feedback and a change of coordinates. An input-output linearizable system is a system with an actual output, whose input-output map can be made linear and the closed loop system state space equations partially linear. In a fully linearizable system both the state space equations and the input-output map can be made linear. It should be stressed that flatness and feedback linearization are in principle different properties which, under certain circumstances, coincide. Namely, a system can be flat in a subset of the state space with no equilibrium point inside (see [111], [90], [91]) whereas linearization only makes sense in the neighbourhood of such a point.

In the latter case one property implies the other. It may seem that this distinction is useless in practice, but there do exist systems with partial equilibrium points. In other words, only part of the state variables eventually reach a stable finite value (see [92], [93], [94]). The mathematical model of bioreactors in fed-batch mode is one example. Despite this fact, flatness is still a very useful concept since it allows to compute algebraically a feedforward control and indicates the system has a very particular structure.

The equivalence of flatness to linearization was established in [87]. In the process, a new kind of dynamic feedback was defined. Namely, the *endogenous feedback*, that will be treated in some detail in a later section. Posterior generalizations, in which the use of time scalings was allowed, led to the concept of *orbital flatness*. The more restricted idea being referred to as *differential flatness*. The connections between these concepts is summarized in figure (A.2).



**Fig. A.2.** Classification of nonlinear systems according to their linearizability



This report is centred on the case of differential flatness. The use of time-scalings is reported in [96] to [101]. It should be noted that many of the basic definitions apply to both cases as they stand in the text.

## A.2 Flatness

One of the main questions underlying flatness theory is: When can one solve an *underdetermined* (system of) ODE(s) *without using integration*? That is to say, When is it possible to parametrize all solutions by a set of arbitrary functions? According to [82] the geometer D. Hilbert was the first one to pose this question in 1912. In particular for ODEs of the form

$$F(t, x, y, \dot{x}, \dot{y}, \dots) = 0 \quad (\text{A.1})$$

For example, the equation

$$(\dot{x})^2 + (\dot{y})^2 = 1 \quad (\text{A.2})$$

can be solved using integration by

$$x(t) = \int \cos \theta(t) dt \quad y(t) = \int \sin \theta(t) dt \quad (\text{A.3})$$

but also, using the following expressions,

$$\begin{aligned} t &= \ddot{f}(\alpha) + f(\alpha) \\ x &= \cos(\alpha) \dot{f}(\alpha) + \sin(\alpha) \ddot{f}(\alpha) \\ y &= \sin(\alpha) \dot{f}(\alpha) - \cos(\alpha) \ddot{f}(\alpha) \end{aligned} \quad (\text{A.4})$$

where  $\alpha$  is a parameter and  $f$  an arbitrary function. It can be checked that

$$\frac{dx}{dt} = \frac{\frac{dx}{d\alpha}}{\frac{dt}{d\alpha}} = \cos(\alpha) \quad \dots \quad (\text{A.5})$$

Hilbert also showed that such formulas do not exist for

$$\dot{x} = (\dot{y})^2 \quad (\text{A.6})$$

Hence, the answer to the question at the beginning is not always positive. In [85], (differentially) flat systems are defined as underdetermined systems

of (nonlinear) ordinary differential equations (ODEs) whose solution curves are in smooth one-one correspondence with arbitrary curves in a space whose dimension equals the number of equations by which the system is underdetermined. Control systems are a natural example of underdetermined systems, i.e. systems with more dependent variables (usually divided into “inputs” and “states”) than equations.

A general underdetermined system of ODEs of order  $k$  may be written as

$$F^j(t, x, x^{(1)}, \dots, x^{(k)}) = 0, \quad j = 1, \dots, N - p. \quad (\text{A.7})$$

where  $F^j$  are assumed to be  $C^\infty$ -smooth functions,  $x = (x_1, \dots, x_N)$  are the dependent variables,  $x^{(r)}$  is the  $r$ -th time derivative of  $x$ , and  $p \geq 1$  is the number of equations by which the system is underdetermined. In the case of control systems,  $p$  coincides with the number of inputs. If any  $p$  dependent variables are set to arbitrary functions of time, then a fully determined system of ODEs is obtained. If in a control system the inputs, say  $x_{N-p+1} = u_1, \dots, x_N = u_p$ , are set to arbitrary functions then a system is obtained whose solutions depend on  $N - p$  constants (i.e. the initial conditions). But there is no reason to assign the arbitrary functions of time to the inputs. Any  $p$  dependent variables may be chosen. Actually it may be possible to choose  $p$  functions of the dependent variables and a finite number of their derivatives as “free variables”. Are then the initial conditions always necessary to parametrize the entire set of solutions or it may suffice with those  $p$  “free variables”?

Whenever the second possibility is true, the system is said to be (diferentially) flat. A formal definition could be the following:

*The system given by eq. (A.7) is said to be differentially flat if there exist variables  $y_1, \dots, y_p$  given by an equation of the form*

$$y = h(t, x, x^{(1)}, \dots, x^{(m)}) \quad (\text{A.8})$$

*such that the original variables  $x$  may be recovered (locally) from  $y$  by an equation of the form*

$$x = \phi(t, y, y^{(1)}, \dots, y^{(l)}). \quad (\text{A.9})$$

*The variables  $y_1, \dots, y_p$  are referred to as the “flat outputs”.*

Let's particularize this definition for control systems (and differential flatness). If the states are  $x_1, \dots, x_{N-p} = x_n$  and the inputs are taken as  $x_{N-p+1} = u_1, \dots, x_N = u_p$ , then, for a system in the form

$$\dot{x} = f(x, u) \quad (\text{A.10})$$

the definition of (differential) flatness is as follows [102]:

“The system with  $m$  inputs  $u$  and  $n$  states  $x$

$$\dot{x} = f(x, u)$$

is said (differentially) flat if there exists a set of  $m$  variables

$$y_i = h_i(x, u, \dot{u}, \dots, u^{(\gamma_i)}) \quad i = 1, \dots, m.$$

such that

- (i) the  $m$  components of  $y$  are differentially independent,
- (ii) the state  $x$  and the input  $u$  can be expressed as functions of  $y$  and its derivatives in finite number :

$$\begin{aligned} x &= \Phi(y, \dots, y^{(\nu)}) \\ u &= \Psi(y, \dots, y^{(\nu+1)}) \end{aligned}$$

with  $\Phi$  and  $\Psi$  identically satisfying  $\dot{\Phi} = f(\Phi, \Psi)$ ”.

A system is said to be *r-flat* if it admits a flat output depending on derivatives of  $u$  of order at most  $r$ , i.e:

$$y = h(x, u, \dot{u}, \dots, u^{(r)}) \tag{A.11}$$

As mentioned above, Fliess and coworkers proved the equivalence of feedback linearization and flatness. They did so using the mathematical framework of infinite-dimensional differential geometry or more precisely “differential geometry of jets and prolongations of infinite order”. In this setting, a system (A.10) is regarded as an infinite family of vector fields parametrized by  $u$  and integral curves are described as smooth functions  $t \mapsto (x(t), u(t))$  with initial conditions in the form of the infinite sequence  $\xi_0 = (x_0, u_0, \dot{u}_0, \ddot{u}_0, \dots)$ . Therefore, all objects are defined using the infinite sequence of coordinates  $\xi = (x, u, \dot{u}, \ddot{u}, \dots) \in X \times U \times R_m^\infty$  where  $R_m^\infty = R^m \times R^m \times \dots$ . In this context, a smooth function is a function smoothly depending on a *finite* but arbitrary number of coordinates. If the original vector field in (A.10) is prolonged as

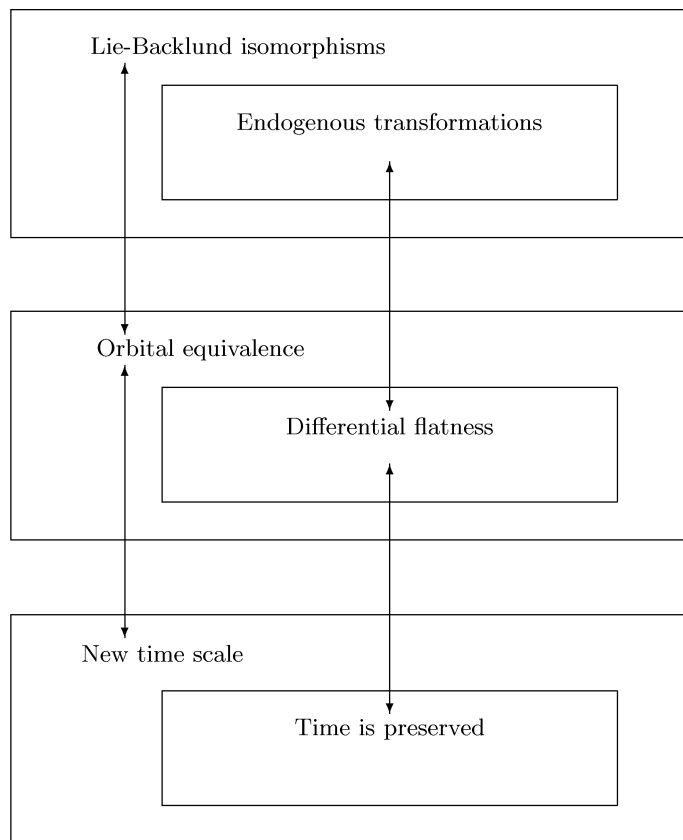
$$F(\xi) = (f(x, u), \dot{u}, \ddot{u}, \dots) \tag{A.12}$$

then equation (A.10) reads

$$\dot{\xi} = F(\xi) \quad \xi(0) = \xi_0 \tag{A.13}$$

Therefore, equation (A.13) defines a vector field, in the classic sense, on the infinite dimensional manifold  $X \times U \times R_m^\infty$ . (See [118], [119], [120]).

Two systems are “equivalent” if there is an invertible transformation exchanging their trajectories. That is, if there exists a smooth invertible mapping  $\Phi$  that takes every integral curve of (A.13) (defined in a neighbourhood of a point  $p$  in the corresponding infinite-dimensional manifold  $M$ ) into an integral curve of another system  $\dot{\zeta} = G(\zeta)$  (defined in a neighbourhood of a point  $q = \Phi(p)$  in the corresponding infinite-dimensional manifold  $N$ ), then both systems are (locally) equivalent. Vector fields  $F$  and  $G$  are said to be related. The mapping  $\Phi$  is called an *endogenous transformation*. This definition can be extended to the time-varying case and to the case of orbital flatness, with endogenous transformations replaced by the more general notion of *Lie-Bäcklund isomorphisms*. (See [120]).



**Fig. A.3.** Lie-Bäcklund isomorphisms and Endogenous transformations

The above mentioned one-to-one relation between trajectories implies that the variables of one system can be expressed as a function of the second system variables and a finite number of their derivatives (See [88]).

Within this mathematical framework, a series of important facts have been established [88]:

- Lie-Backlund isomorphisms and endogenous transformations preserve the number of input channels. Hence, if two systems are orbitally or differentially equivalent then they have the same number of inputs.
- Consider a flat system, i.e. one which is orbitally or differentially equivalent to a trivial system (a linear controllable system in Brunovski canonical form). The number of flat (or linearizing) outputs is equal to the number of input channels.
- If a nonlinear system (A.10) is differentially flat around a point  $p$ , then it satisfies the strong accessibility property at  $p$ .
- If two systems

$$\dot{x} = f(x, u) \quad \text{or} \quad (X \times U \times R_m^\infty, F) \quad (\text{A.14})$$

and

$$\dot{y} = g(y, v) \quad \text{or} \quad (Y \times V \times R_s^\infty, G) \quad (\text{A.15})$$

are differentially equivalent then there exists an endogenous dynamic feedback

$$\begin{aligned} u &= \sigma(x, z, w) \\ \dot{z} &= a(x, z, w) \end{aligned} \quad (\text{A.16})$$

such that the closed loop system (A.14)-(A.16) is diffeomorphic to (A.15) prolonged by sufficiently many integrators. That is, diffeomorphic to

$$\begin{aligned} \dot{y} &= g(y, v) \\ \dot{v} &= v^{(1)} \\ \dot{v}^{(1)} &= v^{(2)} \\ &\vdots \\ \dot{v}^{(\mu)} &= w \end{aligned} \quad (\text{A.17})$$

for  $\mu$  large enough. Consequently, if a system is differentially flat then there exists an endogenous dynamic feedback such that the closed loop system is diffeomorphic to a linear controllable system.

The proofs of these statements, and in particular the relation between equivalence and feedback, can also be found in [127].

It is interesting to analyze the last statement. A linear controllable system is equivalent to the Brunovsky Canonical Form. Brunovsky [103], showed that any controllable linear system

$$\dot{x} = Ax + Bu \quad (\text{A.18})$$

with  $x \in R^n$  and  $u \in R^p$  can be converted, via a linear state transformation and a linear feedback, into a canonical form given by  $p$  chains of integrators:

$$\begin{aligned} \dot{x}_1^1 &= u^1 & \dots & \dot{x}_1^p = u^p \\ \dot{x}_2^1 &= x_1^1 & \dots & \dot{x}_2^p = x_1^p \\ &\vdots & & \vdots \\ \dot{x}_{k_1}^1 &= x_{k_1-1}^1 \dots x_{k_p}^p & = & x_{k_p-1}^p \end{aligned} \quad (\text{A.19})$$

with  $n = k_1 + \dots + k_p$ .

If  $p$  arbitrary functions are assigned to the  $p$  states  $x_{k_1}^1 \dots x_{k_p}^p$  then, it is possible to get all the other states and inputs by successive differentiation. From this observation, it is possible to get back to the original definition of flatness.

There are other ways of attacking the equivalence problem. In the 1910s and 1920s the geometer Elie Cartan developed a set of tools for the study of equivalence of systems of differential equations [105], [106], [83]. These tools are now set in the context of Exterior Differential Systems (See [109]) and make use of Exterior Algebra and Calculus of forms [107]. For example, in this setting a control system is expressed as a *Pfaffian system*:

$$\begin{aligned} \dot{x} = f(x, u) & \quad \mapsto \quad I = (dx_i - f_i(x, u)dt) \\ x \in M, u \in R^m & \quad (x, u, t) \in M \times R^m \times R \end{aligned} \quad (\text{A.20})$$

Many other mathematical objects such as vector fields, distributions... have also their counterparts in this setting (See [108]). In [110] there is a summary of the relation between flatness and Cartan's concept of absolute equivalence. It is also shown that endogenous feedback has a counterpart in the framework of EDS, namely the *Cartan prolongations*. Some of the advantages of this approach are, in principle, the availability of tools from advanced algebra and the fact that implicit equations and non-affine systems can be treated in a unified framework. Unfortunately these techniques are complex (see [109], [104]) and completely unfamiliar to an engineer.

Finally, some researchers have established the relation between system equivalence and system symmetries (See [116], [117]). From this standpoint they have established necessary and sufficient conditions for flatness. Nevertheless, for the time being, no efficient method for checking them and computing flat outputs has been found.

### A.3 Endogenous Feedback.

In the framework of flatness theory, Fliess and coworkers introduced the concept of *endogenous feedback*. The objective of this section is to define it, place it among the other kinds of feedback and give a definite idea of its possible actual realizations.

Given a control system  $\dot{x} = f(x, u)$ , a dynamic feedback

$$\begin{aligned} \dot{z} &= a(x, z, v) \\ u &= b(x, z, v) \end{aligned} \tag{A.21}$$

is said to be endogenous if

- it is regular.
- $z$  and  $v$  satisfying (A.21) can be expressed as functions of  $x, u$  and a finite number of their derivatives:

$$\begin{aligned} z &= \alpha(x, u, \dots, u^{(l)}) \\ v &= \beta(x, u, \dots, u^{(l)}) \end{aligned} \tag{A.22}$$

A slightly different definition is given in [111], allowing for explicit time dependence of functions  $a, b, \alpha$  and  $\beta$ .

Note the endogenous feedback does not add *new dynamics*, in the sense that it does not contain exogenous variables (i.e. independent of the original system variables and their derivatives). Hence the name.

The transformations operated by an endogenous feedback can be undone by another such feedback. Controllability is one of the properties preserved by this kind of equivalence. As remarked in [87] and [127], it is worth pointing that a feedback which is invertible in the standard sense [124] is not necessarily endogenous. The invertible feedback

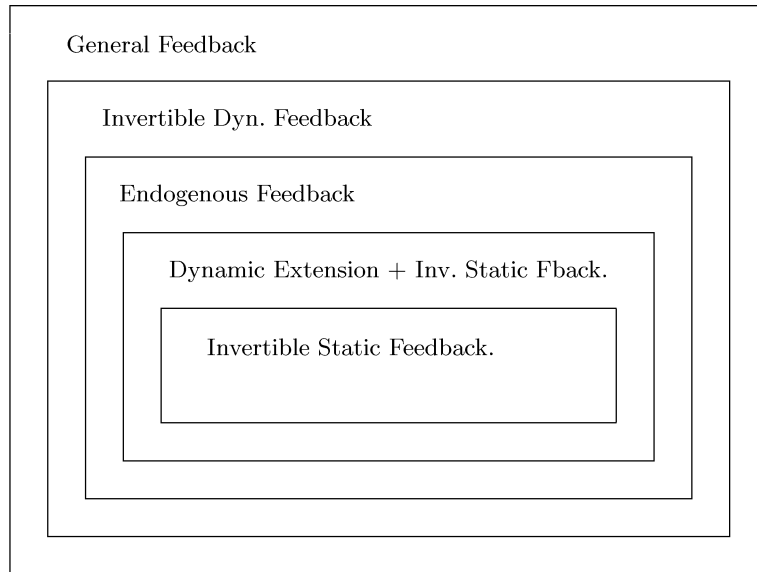
$$\dot{z} = v \quad u = v \tag{A.23}$$

acting on the scalar dynamics  $\dot{x} = u$  is not endogenous and the closed loop

$$\dot{x} = v \quad \dot{z} = v \tag{A.24}$$

is no longer controllable. A diagram showing the relationship between different kinds of feedback is given in figure A.4. The term *dynamic extension* means that chains of integrators have been added to the inputs thus creating new states which are derivatives of the original inputs.

Another example of feedback which is not endogenous is given in [111]:



**Fig. A.4.** Classification of Feedback

$$\begin{aligned}
 \dot{z}_1 &= z_2 \\
 \dot{z}_2 &= -z_1 \\
 u &= g(z)v
 \end{aligned}
 \tag{A.25}$$

Then, how may an endogenous feedback look like?

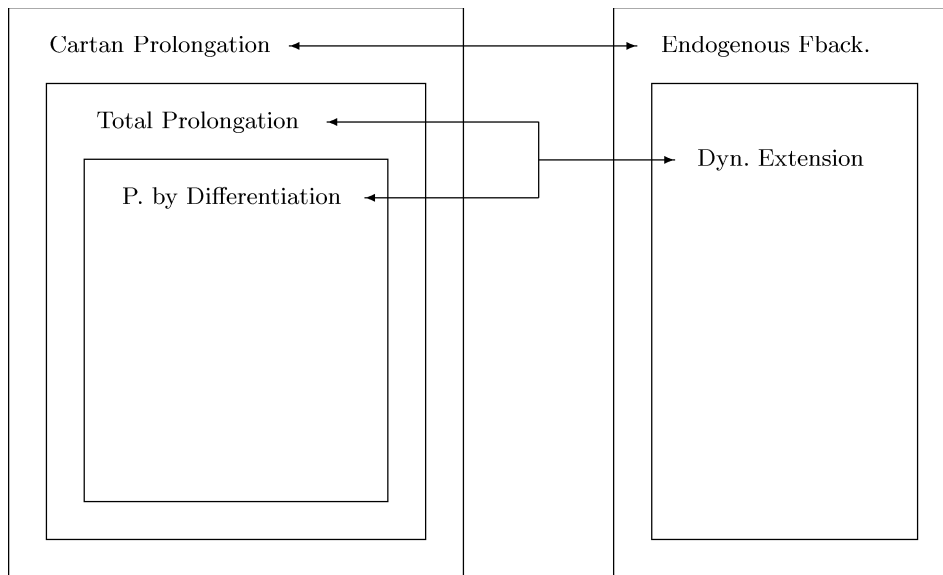
In [121] (and also in [84]) a precompensator (e.g. a dynamic extension) is added to the original system and then it is checked whether the compound system is linearizable using static feedback and a change of coordinates. In particular, in [121] it is suggested that a rather general kind of endogenous feedback may be formed by:

- 1- A dynamic state feedback of the form  $v = \alpha(x, u, \dot{u}, \dots, u^{(\beta)})$ .
- 2- A dynamic extension on the new control variables  $v$ .
- 3- A (nonlinear) static feedback.

In [84] two examples are given which correspond to this scheme. One of them coincides with the PVTOL which in [87] is proved to be flat and equivalent to other systems.

Finally, it is interesting to note that the concepts of dynamic extension and endogenous feedback have their counterparts in the framework of exterior differential systems. See figure A.5 and the article [111].





**Fig. A.5.** Cartan Prolongations and Endogenous Fback.

## A.4 Application to Bioreactors.

In this section the 0-flatness of the standard bioreactor model is studied under several assumptions on the available inputs and the possible flat outputs. The study is carried out “by inspection”. As explained in the previous section *Exact Linearization and Flatness*, in general there is no systematic method for checking flatness and finding the flat outputs [128]. The few existing methods only work for particular cases and entail complex mathematics. See [131], in which 0- and 1-flatness of affine systems with two inputs and four states is treated, for a good example of the complexity of checking r-flatness even for r-small. In this report both infinite-dimensional differential geometry and concepts of exterior differential systems are combined. In the future it may be interesting to deal with this kind of techniques. For the time being, as already said above, a more basic study is carried out.

### A.4.1 0-flatness

To begin with, let’s remind the definition of zero-flatness:

*A system is 0-flat if the flat outputs only depend on the system states and inputs*

In order to understand the method used for checking it, it must also be remembered that the set of differential equations can be regarded as a set

of algebraic equations with variables  $x, s, \dots, \dot{x}, \dot{s}, \dots, u_i$ . If  $x = x(t)$  is assumed to be known, then clearly  $\dot{x}(t)$  is also known. But  $s$  and  $\dot{s}$  count as different incognita. Then it must be checked if there are as many equations as incognita. In case more (algebraically independent) equations are needed, it is possible to generate them by differentiation with respect to time of the existing ones. When choosing the equation to be differentiated it is important to check no new incognita are generated in the process, e.g.  $\dot{u}_i$  or  $\ddot{s}$ . Exceptionally, it may happen that the resulting equation is a combination of the existing ones. So, in the end, independence of all equations should be checked anyway. Maybe, it is also possible to check for 1-flatness in this way but becomes very difficult.

Following the method described above the 0-flatness of the simplest bioreactor was determined [126].

$$\begin{aligned}\dot{x} &= \mu(s)x - Dx \\ \dot{s} &= -y_s\sigma(s)x + D(s_i - s) \\ \dot{v} &= F \quad D = \frac{F'}{v}\end{aligned}\tag{A.26}$$

In that article it was assumed that  $\sigma = \mu$ , but this doesn't affect the reasoning. On the other hand, it is still assumed that  $\mu$  is monod-like. If  $(F, s_i)$  are taken as inputs and  $(x, v)$  as flat outputs, then

- From the first and third equations in (A.26)

$$s = \frac{k_s}{\frac{\mu_m x v}{x v + \dot{v} x} - 1}\tag{A.27}$$

- From the third equation

$$F = \dot{v}\tag{A.28}$$

- And from these and the second one it can be deduced that  $s_i$  is a function of  $(x, v, \dot{x}, \dot{v}, \ddot{x}, \ddot{v})$ .

Since  $x$  and  $v$  are measurable, it would be possible to use a control scheme such as that suggested in [125] or in [126] in a version combined with fuzzy control methods. It is also possible to determine 0-flatness via an already established result (see [128], [130]), according to which any affine system with  $n$  states and  $n-1$  inputs is 0-flat as soon as it is controllable or more precisely strongly accessible. But system (A.26) should be put first in affine form. If the system is expressed using absolute masses instead of concentrations

$$\begin{aligned}\dot{\bar{x}} &= \mu\bar{x} \\ \dot{\bar{s}} &= -y_s\sigma\bar{x} + F s_i \\ \dot{v} &= F'\end{aligned}\tag{A.29}$$

it is clear that the substrate supplied to the bioreactor is equal to  $Fs_i$ , where  $u_1 = F$ ,  $u_2 = s_i$ . In practice this is equivalent to  $Fs_i = F_{max}s_{imax} + F_{min}s_{imin}$  where the new control inputs are  $u_1 = F_{max}$ ,  $u_2 = F_{min}$  and  $s_{imax}$ ,  $s_{imin}$  are constants.

#### A.4.2 Applications

Remember the equations of the second type standard model

$$\begin{aligned}
\dot{x} &= \mu(s, p)x - Dx \\
\dot{s} &= -y_{x/s}\mu(s, p)x - y_{x/p}\pi(s, p)x + D(s_{in} - s) \\
\dot{p} &= \pi(s, p)x - Dp \\
\dot{v} &= F \quad D = \frac{F}{v}
\end{aligned} \tag{A.30}$$

where the control actions are  $u_1 = F$ ,  $u_2 = s_{in}$ , or

$$\begin{aligned}
\dot{x} &= \mu(s, p)x - Dx \\
\dot{s} &= -y_{x/s}\mu(s, p)x - y_{x/p}\pi(s, p)x + D(s_{in} - s) \\
\dot{p} &= \pi(s, p)x - \alpha p - Dp \\
\dot{v} &= F \quad D = \frac{F}{v}
\end{aligned} \tag{A.31}$$

where the control actions are  $u_1 = F$ ,  $u_2 = \alpha$ .

As a rule these models are not flat with  $x$  and  $v$  as flat outputs, except for the second case with  $\mu_1 = \mu_1(s)$ . Then, it is 0-flat (at least locally):

- From the first and fourth equations  $s = f(x, v, \dot{x}, \dot{v})$ .
- Now the known variables are  $x, v, s, \dot{x}, \dot{v}, \dot{s}, F$ .
- From the second equation,  $p$  and then  $\dot{p}$  are obtained.
- Finally, from the third equation  $\alpha$  is obtained.

If  $\mu_1 = \mu_1(s, p)$  that's no longer possible. After a series of attempts, it was found out that the total biomass  $\bar{x} = xv$  and the total product mass  $\bar{p} = pv$  can be used as flat outputs when using  $(F, s_{in})$  as inputs. Expressing the system, not in concentrations but in absolute masses, and making it affine through  $(F, s_{in}) \mapsto (F_1, F_2)$  :

$$\begin{aligned}
\dot{\bar{x}} &= \mu(\bar{s}, \bar{p}, v)\bar{x} \\
\dot{\bar{s}} &= -y_{x/s}\mu\bar{x} - y_{x/p}\pi\bar{x} + F_1s_{inmax} + F_2s_{inmin} \\
\dot{\bar{p}} &= \pi(\bar{s}, \bar{p}, v)\bar{x} \\
\dot{v} &= F_1 + F_2
\end{aligned} \tag{A.32}$$

it can be checked that the system is (at least locally) 0-flat with  $(\bar{x}, \bar{p})$

- The first and third equations constitute a system of two equations with two incognita  $\bar{s}, v$ . Then, it is possible to obtain these two variables and consequently  $\dot{\bar{s}}, \dot{v}$ .
- Substituting in the second equation, the only incognita left are  $F_1, F_2$ . Hence, taking also the fourth equation, there appears another system of two equations with two incognita.

The same reasoning also works in the non-affine case and even in the more general case. On the other hand, it turned out that with  $(F, \alpha)$  as inputs, the system is not 0-flat.

There are other two possible alternatives:

- *Use dilution instead of flux as control action .*  
Work with the equations in concentrations and take as inputs  $(D, s_{in})$  (equivalently  $(D_1, D_2)$ ) or  $(D, \alpha)$ . In this way, it is possible to disregard the equation of volume. Thus, obtaining an affine system with three states and two inputs which is always flat, if it is controllable. Afterwards, once  $D$  has been determined and knowing  $v_{(t=0)}$ , it is possible to determine  $F$ .
- *Fix volume trajectory (or equivalently  $D=D(t)$ ).* From the very beginning, fix the trajectory of volume and consequently that of  $F$ . Then take as control actions  $(\alpha, s_{in})$ . This would require two precision pumps for  $s_{in}$  and one standard pump for  $\alpha$ , but on the other hand fermentations would have a definite and precise duration. An affine time-varying system with three states and two inputs is obtained. It should be checked that is 0-flat.

These two alternatives could be useful in order to reduce the possible systems with five states and two inputs to the case of affine systems with four states and two inputs.

It should be noted that in continuous mode fermentations the standard model always reduces to the three states-two inputs case.

## B Additional documentation

*Letter to the Editor of Biotechnology Progress.*

The above paper<sup>1</sup> deals with the control of the substrate concentration of fed-batch bioreactions with non-monotonic growth kinetics. Firstly, a straight-forward feedback linearization control strategy is introduced which assumes that both biomass and substrate concentrations are available for feedback. However, as the authors assert in section 3.6 of their paper, this assumption is generally not verified because on-line substrate concentration measurements are not available and observers based on biomass concentration are not a valid alternative for stabilization of non-monotonic fed-batch processes. To overcome this drawback, a modified version of this preliminary linearizing control strategy is presented which is much more realistic from a practical viewpoint. Its main advantage is that the implementation only requires the measurement of biomass concentration and the estimation (based on this measure) of the specific growth rate. Then, the authors attempt to demonstrate that the fed-batch process can still be stabilized around any desired set-point all along the non-monotonic kinetics by introducing a discontinuity in the feedback gain. Unfortunately, the stability analysis developed by the authors is not entirely consistent with the proposed control law based only on biomass measurement. So, the claimed demonstration of global stability without feedback of the substrate concentration is not completely valid. In this context, this note is aimed at clarifying some points of this demonstration and at showing that the control law proposed by the authors effectively stabilizes the substrate concentration at any set-point on the non-monotonic kinetics.

Note that replacing the control law (5) in the mass balance equation (2) does not yield the linear dynamics (4) (This can also be verified in Figure 2 which does not display the typical exponential response of linear systems). Similarly, the control law (6) does not lead to the closed-loop dynamics (7) (Actually, to obtain the closed-loop dynamics (7), the set-point  $C_S^*$  should be replaced by the actual substrate concentration  $C_S$  in the denominator of

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<sup>1</sup> I.Y. Smets, G.P. Bastin and J. Van Impe, Feedback Stabilization of Fed-Batch Bioreactors: Non-Monotonic Growth Kinetics, *Biotechnol. Prog.* 2002, 18, 1116-1125.

both terms in the right-hand side of (6), but this correction would introduce feedback of  $C_S$  and the most attractive feature of this control strategy would be lost). Consequently, the stability analysis developed from (7) in section 3 requires some corrections to effectively demonstrate that the control law (6) as proposed by the authors (i.e. without on-line measurement or estimation of  $C_S$ ), globally stabilizes the system at any desired set-point  $C_S^*$  all along the non-monotonic kinetics. We give here some guidelines for this demonstration and derive some necessary and sufficient conditions.

First of all, it is convenient to reformulate the gain  $\tau_\mu$  in (6) as a linear function of biomass:  $\tau_\mu = kC_X/Y_{X/S}$ . Also, after some trivial algebra, the growth rate error  $\mu - \mu^*$  for a Haldane kinetics can be written in (6) as

$$\mu - \mu^* = -\frac{\mu}{C_S} \frac{1}{b} (C_S - C_S^*) (C_S - C_{S,a}^*)$$

where  $b = K_I \mu_m / \mu^*$ . Then, replacing (6) in the mass balance equation (2), the following closed-loop dynamics yields

$$\frac{d(C_S - C_S^*)}{dt} = - \left( m + \frac{1}{Y_{X/S}} \frac{\mu}{C_S} g(C_S) \right) \frac{C_X}{(C_{S,in} - C_S^*)} (C_S - C_S^*)$$

where the function  $g(C_S)$  is given by

$$g(C_S) = C_S + \frac{k}{b} (C_S - C_{S,in}) (C_S - C_{S,a}^*)$$

For the sake of simplicity, the stabilizing maintenance coefficient  $m$  is hereinafter neglected. Then, to accomplish global stability at any  $C_S^*$ ,  $g(C_S)$  must be strictly positive for all achievable values of  $C_S$ , i.e.  $\forall C_S \in [0, C_{S,in}]$ . After some manipulation, the quadratic polynomial  $g(C_S)$  can be written as

$$g(C_S) = ak \left[ \frac{C_S^2}{C_{S,in} C_{S,a}^*} - \frac{C_S}{C_{S,in} C_{S,a}^*} \left( (C_{S,in} + C_{S,a}^*) - \frac{b}{k} \right) + 1 \right], \quad a > 0.$$

Clearly, the necessary and sufficient conditions for global stability are:

- $g(0) > 0$ .
- $g(C_S)$  has no root in  $[0, C_{S,in}]$ .

To satisfy these stability conditions, the gain  $k$  should be selected within the range<sup>2</sup>

<sup>2</sup> Actually, due to the presence of the maintenance coefficient, stability is achieved with a larger range of  $k$ , in particular with  $k = 0$ , i.e. the open-loop control (3) of the above paper also stabilizes the system.

$$0 < k < \bar{k} = \frac{b}{C_{S,in} + C_{S,a}^* - 2\sqrt{C_{S,in}C_{S,a}^*}}$$

In fact, selecting  $k < 0$  violates the stability condition  $g(C_S) > 0$  for low values of  $C_S$  (below the left flank equilibrium), whereas selecting  $k > \bar{k}$  violates the condition  $g(C_S) > 0$  for high values of  $C_S$  (above the right flank equilibrium).

Remark 1: A performance analysis reveals that growth rate error feedback with  $k > 0$  improves the local convergence to a set-point on the left flank of the Haldane kinetics, whereas deteriorates the local response around a set-point on the right flank. Actually, to fasten the convergence towards this latter set-point, the gain  $k$  should be negative, but the response from low initial substrate concentrations might be unstable.

Remark 2: If appropriate discontinuous feedback is introduced, for instance replacing  $k$  by  $|k|sign(C_{S,a} - C_S)$  in the expression of  $\tau_\mu$ , then the stability condition  $g(C_S) > 0 \forall C_S \in [0, C_{S,in}]$  is verified for all  $k$ , hence for all  $\tau_\mu$  (positive or negative). This discontinuity is equivalent to the switching factor introduced by the authors to achieve stability. It is shown here however that including discontinuous feedback is not a necessary condition to guarantee stability. Anyway, it is useful to improve the closed-loop performance, particularly for operation at high substrate concentration levels.





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