STUDIES ON MODELING AND CONTROL OF CONTINUOUS BIOTECHNICAL PROCESSES

by

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In memory of my grandmother, Youlian Huya

on her centennial birth anniversary

纪念我的外祖母，胡雅友莲

一百周年诞辰

ABSTRACT

Continuous bioreactors are gaining considerable industrial importance as they provide better utilization of the equipment and improvement of the productivity. On the other hand, as for batch and fed-batch bioprocesses, the inherent on-line measurement difficulties and complex dynamics with biological systems pose great obstacles to the development of advanced control strategies for continuous bioreactors. Therefore, the field of continuous bioprocess control offers a wide range of research activities and challenges to control engineers. An attempt is made in this thesis to find more efficient control systems which provide quality assurance and economic incentives for the operation of continuous bioprocesses in the face of these two bottlenecks above mentioned.

In this thesis, simple frequency-dependent tools such as the relative gain array, the closed loop disturbance gain, and the partial disturbance gain are used for controllability analysis with respect to disturbance rejection and control structure selection. In particular, the partial disturbance gain introduced by Skogestad and Wolff (1992) is generalized to the common case with more outputs uncontrolled. This lays the foundation for control system design work presented in the thesis. The thesis also demonstrates that the partial disturbance gain is an effective tool for controllability analysis, by virtue of its independence of the controller design, and one avoids time consuming on detailed time-response simulations.

The first major part of this thesis is on the evaluation of five control configurations for continuous bioreactors, based on the controllability analysis with respect to the disturbance rejection by using the partial disturbance gain.

A large effort in this thesis is made in the extensive study of an industrial process, high concentration cultivation of *Propionibacterium shermanii* in a continuous bioreactor with cross-flow filtration. Studies on this industrial process cover the topics of Modeling, Estimation and Control.

Although the nutrition and metabolism of propionibacteria have been studied extensively, very little quantitative information is available in the literature about the fermentation kinetics of Propionibacteria growth limited by a complex substrate, yeast extract-peptone, especially with regard to the saturation constant (K_s) in the Monod model. To fill this void, a preliminary study on the cell growth kinetics is made, and some estimates for the parameters μ_m and K_s in the Monod model are obtained from these experimental results. Furthermore,

simulation results show by a sensitivity analysis that a high K_s-value has a big effect on the steady state behavior of the process, and thus stress the importance of the knowledge about K_s for rational operation of this bioreactor.

To cope with the on-line measurement difficulties, an approach that incorporates material balance model with the extended Kalman filter is used. A dynamic model is developed that provides relations between cell growth rate and two environmental variables, i.e. base addition rate and dissolved CO_2 concentration which are on-line measurable. Simulation results demonstrate the possibility to on-line estimate the cell concentration and other state variables from these two available on-line measurement variables by applying the extended Kalman filter.

Aiming at the control of this continuous bioreactor, at first by using simple frequency-dependent tools such as the relative gain array and the closed loop disturbance gain, the issues on the selection of manipulated inputs and how to pair the controlled outputs with manipulated inputs for 3×3 decentralized control of this bioreactor are discussed. Nevertheless, based on the controllability analysis with respect to disturbance rejection by using the partial disturbance gain, a 1×1 partial control configuration is proposed as the most efficient and practical way to control this continuous bioreactor, in that a more easily on-line measured process variable is selected as the controlled variable and the main operation objective is achieved.

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Chapter 1

Introduction

1.1 Background - What Is Biotechnology?

In 1982 the Organization for Economic Co-operation and Development (OECD) proposed the following definition of biotechnology (Bull et al., 1982), which is the best-known and most widely accepted definition today:

"*BIOTECHNOLOGY* is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services".

In this definition "scientific and engineering principles" covers a wide range of disciplines but rely heavily on microbiology, biochemistry, genetics, biochemical and chemical engineering; and "biological agents" refers to a wide range of biological catalysts but particularly to microorganisms, enzymes and animal and plant cells. The definition refers to the provision of "goods and services". The former includes the products of industries concerned with food, beverages, pharmaceuticals, and biochemicals; the latter is largely concerned with water purification, industrial and domestic waste management.

Another useful definition was provided by the European Federation of Biotechnology (EFB) in 1981 (Rehm and Reed, 1981) :

"*BIOTECHNOLOGY* is the integrated use of biochemistry, microbiology and chemical engineering in order to achieve the technological (industrial) application of the capacities of microbes and cultured tissue cells." Here *Integration* and *application* are the keywords which can be found in most definitions of Biotechnology (see Figure 1.1)

The historical development of biotechnology may be divided into four different phases (see Table 1.1) (Enari, 1982).

Biotechnology started with a long period of traditional fermentations. Many of these processes are familiar to the consumer, e.g. production of beer, wine, vinegar, cheese, yogurt and other fermented dairy products, bread and many oriental or Eastern Asian foods. Alcoholic fermentation was discovered perhaps ten thousand years ago by the Sumerian. About five thousand years later the Egyptians learned to use yeast in baking.

The next period in the history of biotechnology was the development of microbiology which happened about a hundred years ago. Since the important micro-biological discoveries of Pasteur and other scientists at the end of the last century many primary metabolic products have been produced by microorganisms.

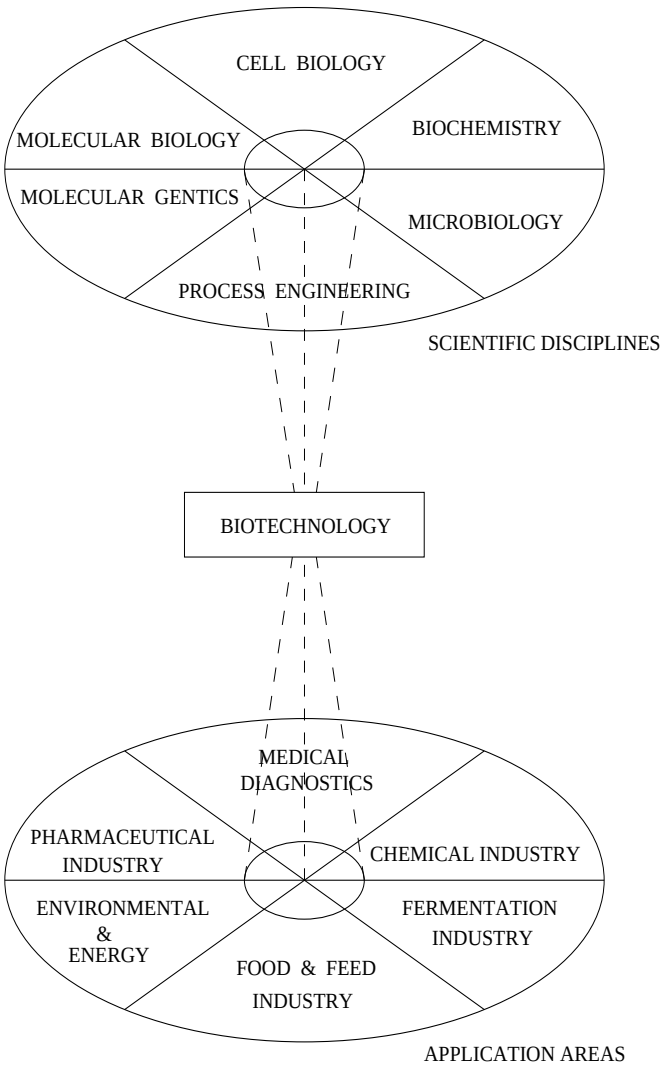

Figure 1.1: Biotechnology: application-oriented integration of biodisciplines and engineering *(Source: reproduced from Riet and Tramper (1991))*

Table 1.1: The History of Biotechnology.

Traditional fermentations	
10 000 B.C.	beer, wine
5 000 B.C.	baking
Microbiology	
1870 - 1890	role of microorganisms
Fermentation technology	
1940 -	large-scale production of penicillin
1950 -	continuous fermentation
1970 -	immobilized enzymes and cells
Information technology	
1970 -	computer technology
1980 -	recombinant DNA technology (genetic engineering)

The next great step was the development of fermenter technology. A major breakthrough in biotechnology occurred in the 1940s as a result of the large-scale production of the first antibiotic, *penicillin*, which necessitated the important engineering developments including the development of techniques for large-scale sterilization, aeration, and growth of microorganisms. Further steps in fermenter technology were the introduction of continuous fermentation ten years later and the invention of immobilized enzymes and cells in the 1970s.

The fourth phase in the history of biotechnology can be called the period of information technology. Computer technology made process control possible in the 1970s. Another part of this period concerns the biological information stored in the hereditary material of the cells. Recombinant DNA technology introduced the possibility of manipulating the genetic properties of microorganisms. The developments in genetic engineering have played a major role in creating the current excitement in biotechnology.

In summary, biotechnology deals with the use of living organisms to manufacture valuable products. As such, it has ancient roots in the agricultural and brewing arts. However, recent developments in genetic manipulative techniques and remarkable advances in bioreactor design and computer-aided process control have founded a "new biotechnology" which considerably extends the present range of technical possibilities and is expected to revolutionize many facets of industrial, agricultural and medical practices. At present, biotechnology is in an amazing growth phase whose end is nowhere in sight. The undisputable fact is that Biotechnology is the technology for the 21st century.

1.2 Motivation

One of the primary objectives of research and developments in the biotechnology industry is the establishment of viable processes through increasing product yields, improving product quality, and reducing production costs. Historically, the most important means of achieving this have been strain improvement using a variety of techniques, growth medium development, and improvements in nutrient feeding. Due to the recent significant efforts made in the measurement of biotechnical variables, bioprocess instrumentation and bioprocess modeling and control, however, potential benefits have been recognized for the application of advanced control techniques. The ability to control biotechnical processes at

their optimal states accurately and automatically is of considerable interest to many fermentation industries since it can enable them to reduce their production costs and increase the yield while at the same time maintaining the quality of the metabolic products. More traditional processes have benefited from these new manufacturing techniques in several ways, such as the use of improved types of equipment, better processing strategies and new methods for bioprocess monitoring and control.

However it should be noted that the control system design of bioreactors is not straightforward due to (Shimizu, 1993):

1) the lack of accurate mathematical models which can describe the cell growth and metabolite production,

2) the lack of reliable on-line sensors which can detect the important state variables, such as the cell concentration,

3) the nonlinear and time-varying properties of the system, and

4) the slow responses of the process in particular for cell and metabolite concentrations.

To cope with the first problem, the control system must be robust with respect to the model uncertainty and must have the ability to reject disturbances. As for the second problem, the effective on-line sensors are limited for use in many cases so that some sort of state observer and/or parameter estimator needs to be designed from the available measurement variables. As for the third problem, the nonlinear control needs to be developed to cope with the bioprocess nonlinearity; and also the "self-tuning" type of adaptive control may be useful to overcome the time-varying problem of bioprocess. As for the fourth problem, the DMC (dynamic matrix control), which is in the category of predictive control, is particularly well suited to the bioprocess with significant slow response. From the practical application point of view, the optimizing control is quite important.

1.3 Basic Definitions

Plants: A plant is a piece of equipment, perhaps just a set of machine parts functioning together, the propose of which is to perform a particular operation. In this thesis we shall call any physical object to be controlled (such as bioreactor or fermenter) a *plant*.

Processes: The *Merriam-Webster Dictionary* defines a process to be a natural, progressively continuing operation or development marked by a series of gradual changes that succeed one another in a relatively fixed way and lead toward a particular result or end; or an artificial or voluntary, progressively continuing operation that consists of a series of controlled actions or movements systematically directed toward a particular result or end (Ogata, 1990). In this thesis we shall call any operation to be controlled a *process*. Examples are fermentation processes or biotechnological processes.

State: The state of a bioreactor system is defined as the set of dependent variables which adequately describe the situation in the bioreactor at any point in time. In this thesis the most interested or important state variables are cell mass concentration and substrate concentrations.

Operating variables: are those variables which can be manipulated at the will of user so that optimal operation is achieved. The dilution rate and the concentration of the growth-limiting substrate in the feed are such variables which are mostly used.

Culture parameters or growth parameters: are the parameters that describe the state of growth of microorganisms as well as the state of the other processes associated with the process of growth. Such parameters are the specific growth rate, the various yields, and others.

(input-output) Controllability: The ability to achieve acceptable control performance, that is, to keep the outputs within specified bounds or displacements from their setpoints, in spite of unknown variations in the plant (e.g. disturbances and model perturbations) by using available inputs and available measurements. Controllability is an inherent property of the process and is therefore independent of controller tuning. It can only be affected by changing the plant itself, that is, by design modifications (Skogestad, 1994).

Control strategy: The decision on how to control the bioreactor to achieve the operating objective. Several tasks are involved: the selection of control objectives, actuators (inputs) and measurements (outputs), and the choice of controller structure (for example: partial control, decentralized control, multivariable control).

Control configuration: The decision on which manipulated variables should be paired with which measured outputs, i.e. how to pair the actuators and measurements. It is customary in continuous bioreactor to denote the control configuration on the basis of which measured output is controlled. Examples are the turbidostat where the cell mass concentration is controlled and the nutristat where the substrate concentration is controlled. This notation is also used in this thesis.

1.4 Thesis Overview

The thesis consists of 6 separate articles on modeling and control of biotechnical processes. The chapter sequence has been organized to reflect a change of research scope, starting from general considerations on continuous bioreactor control, and focusing on an industrial application of a continuous bioreactor with multiple substrate feeding streams and cross-flow filtration. Studies on this industrial process cover the topics of Modeling, Estimation and Control.

Chapter 2 provides a literature review on the computer application on biotechnological processes. The review given in this thesis is confined to the most important techniques related with the areas of on-line identification of bioprocess and control system design with the emphasis on the control of continuous bioprocesses.

Chapter 3 presents a comparison of five control configurations for an idealized continuous stirred-tank bioreactor (CSTR), based on controllability analysis with respect to disturbance rejection, by the use of a controller-independent controllability measure, the partial disturbance gain. In particular, we have generalized the partial disturbance gain, which was first proposed by Skogestad and Wolff (1992) confined to the case that only one output is uncontrolled with other loops closed, to the common case with more outputs uncontrolled. This work lays the foundation for much of the analysis work presented in the later sections of this chapter and for the partial control system design work presented in Chapter 7.

The second part of the thesis is composed of Chapter 4, Chapter 5, Chapter 6 and Chapter 7 which are devoted to the extensive study of a specific process, Propionibacteria Fermentation.

Chapter 4 starts with a very brief introduction to the basic knowledge of cell growth for understanding biotechnical process dynamic behavior. The general

characteristics of Propionibacteria (genus *Propionibacterium*) are also described briefly. The main part of this chapter is a preliminary study on the kinetics of the cell growth. Two batch fermentation experimental results are presented, and we find that the agreement between the batch experiment results and the calculated data based on the Monod kinetics is satisfactory, i.e. the Monod kinetics can describe adequately the growth course of the microorganism *Propionibacterium shermanii*. In addition, some estimates for the parameters μ_m and K_s in Monod model are obtained from these experimental results. In the literature we found no quantitative data available about the K_s value corresponding to the complex medium yeast extract-peptone as limiting substrate.

Chapter 5 is focused on the modeling work for an industrial propionibacteria fermentation process in a continuous bioreactor with cross-flow filtration. The first sections in this chapter describe in details the characteristics of this industrial process and the derivation of the dynamic model of this process. The Monod parameter K_s's influence on steady state behavior of process is highlighted in this chapter. Simulation results show by a sensitivity analysis the effect of the K_s-value on the steady state behavior of process, and then stress the importance of the knowledge about K_s for rational operation of this bioreactor.

Chapter 6 studies the possibility of using the on-line measurable environmental variables to estimate the unmeasured state variables in propionibacteria fermentation process. A dynamic model is developed that provides relations between cell growth rate and two environmental variables, i.e. base addition rate and dissolved CO_2 concentration which are on-line measurable. Simulation results show that it is a possible way to on-line estimate the cell concentration and other state variables from the available on-line measurements of dissolved CO_2 concentration and base addition rate, by applying the extended Kalman filter.

Chapter 7 deals with the final task for this industrial application, namely to properly control this bioreactor. In this paper simple frequency-dependent tools such as the relative gain array, the closed loop disturbance gain, and the partial disturbance gain are used for control structure selection and controllability analysis with respect to disturbance rejection. The issues on the selection of manipulated inputs and how to pair the controlled outputs with manipulated inputs for 3×3 decentralized control of this bioreactor are discussed. The main consideration and the most interesting work in this paper is the study on the possibility of the partial control of this bioreactor. Based on the controllability analysis with respect to disturbance rejection by using the partial disturbance

gain, a 1×1 partial control configuration is proposed as the most efficient and practical way to control this continuous bioreactor.

Final conclusions and suggestions for future work are presented in Chapter 8.

Presentations

Preliminary versions of some of the chapters in this thesis have been presented as papers at international conferences:

- IFAC Symposium ADCHEM'94, Kyoto, Japan, May 1994, pp. 325–330. (Chapter 3.)
- AIChE'94 Annual Meeting, San Francisco, USA, Nov. 1994, Paper 39d. (Parts of Chapter 6.)
- 6th International Conference on Computer Applications in Biotechnology - CAB6, Garmisch-Partenkrichen, Germany, May, 1995, pp. 344–348. (Parts of Chapter 7.)

In addition to the work presented in this thesis, the author has participated in an industrial research project. Reports on these studies are, however, not included in the thesis because of the confidential factor concerned with the industry company. The author has also cooperated in the following project:
Z.-Q. Wang, S. Skogestad and Y. Zhao, "A comparison of various control structures for continuous bioreactors: Exact Linearization Control", Paper 129g, *AIChE Annual Meeting*, Miami Beach, Nov. 1992.
The author's contribution involved calculation and verification work.

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Chapter 2

Literature Review on Computer Control of Biotechnical Processes

2.1 Introduction

The development of computer control of biotechnological processes commenced during the mid-sixties. Murao and Yamashita (1967) were the first to discuss some kind of supervisory monitoring of fermentations by means of computers. Grayson (1969) described the use of computer control in a fermentation plant with application not only in the pilot plant but also to large-scale fermentors. With recent advances in both computer and biotechnology, the application of on-line computer monitoring and control to increase the productivity of biotechnical processes becomes an increasingly important field, and remarkable progress have been made.

Until recently the biotechnology industry has lagged behind other industrial processes in implementing control and optimization technology. The bottleneck in biotechnological process control is the on-line measurement of controlled process variables. Biotechnical processes are much more complex than other industrial processes as they involve living organisms in which a large number of biochemical reactions and transport phenomena take place. The microorganisms have *intracellular* regulatory mechanisms through which they perform *internal* regulation. Without proper understanding of these mechanisms, we can only manipulate the *extracellular* environment by an *external* control system, hoping to affect *intracellular* mechanisms in order to optimize bioreactor performance.

The review given below is confined to the most important techniques related to the areas of on-line identification of bioprocess and control system design with the emphasis on the control of continuous bioprocesses. A comprehensive overview of the state of the art is given in the book "Measuring, Modelling and Control" edited by Schügerl (1991a). The book covers monitoring of the biotechnical process with sophisticated analytical techniques, use of the resulting data by means of mathematical models, and computer-aided closed loop control for improvement of the productivity of biotechnical processes.

2.2 On-line Identification of Bioprocess

The performance of a biotechnical process depends entirely on the maintenance of a well defined and controlled environment for cell growth and product formation. The first step towards this objective is the effective on-line measurement of key physiological and biochemical parameters and important state variables,

which requires on-line analysis of the medium and cell components and characteristics. However, at present the on-line and *in situ* sensors or analytical techniques are still in many cases restricted to the measurements of temperature, pH, various gas compositions, and flow rates of both liquid and gaseous streams (Saucedo et al., 1995). In other words, the overwhelming majority of these on-line sensors quantify *extracellular* chemical and physical quantities and they are not designed or able to measure *intracellular* compounds (Reardon and Scheper, 1991). The major difficulty in developing on-line sensors results from the peculiar constraints posed by the bioprocess. The probes must be capable of withstanding sterilization if they are inside the sterile boundary, followed by repeated heating and cooling cycles, which definitely affect probe reliability and performance. For on-line analysis of the medium composition outside of the bioreactor, an on-line aseptic sampling system is required that does not compromise the bioreactor's sterility. In addition, probes are subject to surface fouling by the microorganisms. Although much effort and major progress have been made in new sensors and process-related chemical analyzers such as On-Line Flow-Injection Analyzers (FIA) and On-Line High Performance Liquid Chromatography (HPLC) (Schügerl, 1991; Hitzmann et al., 1995; Saucedo et al., 1995), the commercial availability, high reliability and low cost of instruments to on-line measure the important *internal* variables, such as cell mass, substrate, and product concentrations, are still very limited. There is thus an urgent need to provide the necessary information by estimating these primary variables from the related secondary or environmental variables that are simpler and easier to measure, i.e. on-line identification of bioprocess.

The objective of bioprocess identification is to determine what has happened, is happening, and probably will happen in the bioprocess, based on all available knowledge about the bioprocess. The on-line bioprocess identification is a systematic method, which utilizes general knowledge on bioprocess behavior and available on-line measurements of bioprocess in order to produce noise-free, corrected measurements, to estimate non-measurable bioprocess variables (state estimation), and to identify unknown key parameters of a predefined bioprocess dynamic model structure (parameter identification). Most of the estimation techniques described in the literature can be divided into the following three basic schemes:

- Estimation through elemental balances

- Extended Kalman filter
- Adaptive estimator

2.2.1 Estimation through elemental balances

The use of the elemental balances was proposed by Cooney et al. (1977). The basic feature of this technique is to represent the biological conservation of substrate to cell mass by an overall chemical reaction as follows (for simplicity, we consider the case that the only product is cell mass):

$$\underbrace{a\,C_\alpha H_\beta O_\gamma}_{\text{substrate}} + b\,O_2 + c\,NH_3 \rightarrow \underbrace{C_\delta H_\varepsilon O_\zeta N_\eta}_{\text{cell mass}} + d\,CO_2 + e\,H_2O \tag{2.1}$$

The stoichiometric coefficient for cell mass has been normalized to 1. All the chemical formulae are assumed known (α through η are known) and constant. The five unknown stoichiometric coefficients a, b, c, d, and e can be calculated from four elemental balances (C, H, N, O) and two measured variables, OUR (oxygen uptake rate) and CER (carbon dioxide evolution rate), which yield the fifth equation needed. The stoichiometric relationships are then used to calculate various rates such as cell growth rate and substrate consumption rate, and species concentrations such as cell and substrate concentrations. This approach is in principle still valid for bioprocesses involving more substrates and products, but additional measurements are necessary. Due to its simplicity and generality, elemental balancing approach has drawn considerable attention, and various on-line calculations based on elemental balances have been published and reviewed recently by Lim and Lee (1991).

However, since the basis of this approach is mass balance, it is not applicable to the complex bioprocesses involving unidentified reactants or products. This problem was solved by the use of the "empirical reaction subspace" determined via "singular value decomposition" of multiple experimental data (Hamer, 1989; Liao, 1989). It is shown that for fermentation state estimation, use of the empirical reaction subspace not only circumvents the problem caused by incomplete mass balance, but also reduces sensitivity of state estimates to measurement errors (Liao, 1989).

2.2.2 Extended Kalman filter

The material balancing method suffers from the inaccuracies of available instruments, and these measurement errors can have profound effects on the accuracy of the on-line estimates. Thus, a good noise filtration algorithm should be employed to improve the reliability of the estimated values before they are used for control purposes.

The Kalman Filter is the optimal state estimator for a linear system when a model for the system together with the knowledge of certain stochastic properties of measurement and disturbance noises is available. For a nonlinear system this technique requires linearization of the model and is known as the extended Kalman filter (EKF). The EKF structure is well suited for biotechnological systems because of its capability to deal with nonlinear systems and to provide combined state and parameter estimation, which can be very attractive for tackling bioreactor identification and estimation. A general treatment of the extended Kalman filter theory can be found in Anderson and Moore (1979) and Ljung (1979). A number of successful developments and applications of EKF for on-line identification and advanced control of biotechnological process have been reported (e.g. Svrcek et al., 1974; Swiniarski et al., 1982; Stephanopoulos and San, 1981; Stephanopoulos and San, 1984; San and Stephanopoulos, 1984a; Shimizu et al., 1989; Albiol et al., 1993).

In particular, some contributions by Gregory Stephanopoulos and his students San and Grosz should be noted here. At the beginning of 1980's, they attempted the on-line identification of batch, fed batch, and continuous fermentation thoroughly, which resulted in a series of publications (Stephanopoulos and San, 1981; Stephanopoulos and San, 1984; San and Stephanopoulos, 1984a; Grosz et al., 1984; San and Stephanopoulos, 1984b). This is the first time that rigorous identification procedures were applied to bioreactors. First, an expanded fermenter state was used, including the usual state variables (such as cell mass, substrate, and product concentrations) as well as the culture parameters (such as the specific growth rate and the overall substrate and product yields). Second, no growth kinetic models were assumed or required for description of the relationships between the culture parameters and state variables. Third, the elemental and macroscopic balances were used to provide the relations between the important variables and the environmental variables (such as pH, CO_2 and O_2 in the exhaust gas) so that the important state variables and culture parameters

can be estimated from more easily accessible on-line measurements of these environmental variables. The proposed on-line identification methodology was tested with a series of numerical simulations and laboratory experiments. Their results confirmed the superior characteristics of the proposed estimator, and its applicability to on-line bioreactor control as well as basic studies of the characteristic properties of microbial cultures.

2.2.3 Adaptive estimator

It should be noted that the successful application of EKF depends largely on the accuracy of the process model. The use of EKF requires a large design effort and *a priori* estimates of measurement noise and model uncertainty characteristics. The EKF can also suffer from some numerical problems and convergence difficulties due to approximations associated with model linearization.

The concept of an adaptive estimator is attractive because it makes use of easily available on-line process information, yet ensures long-term estimator robustness by incorporating high-level off-line information at a much slower sampling rate. It thus avoids both the critical model linearization step used by the EKF and the need for *a priori* knowledge of model uncertainty. Shioya et al. (1982; 1985) proposed an extended adaptive Kalman filter which uses moving averages and dynamic mass balancing, as well as adaptively changing the system noise covariance matrix according to the prediction error. It was shown that the specific growth rate and cell concentration could be estimated with sufficient accuracy. Dochain and Bastin (1984; 1985) proposed an adaptive nonlinear observer for the estimation of specific growth rate and cell concentration. The key feature of the proposed method is that it does not require any kind of analytical description of the specific growth rate which is simply considered as a completely unknown time varying parameter. The stability and convergence properties of the nonlinear observer were later evaluated by Bastin and Dochain (1986). Ramseier et al. (1993) developed a new adaptive cell mass estimator by incorporating an underlying nonlinear model so as to utilize the best possible *a priori* process knowledge. Only a single parameter is updated whenever a new off-line cell mass measurements is available to match the model to the plant operating characteristics. It was shown by experimental results that such an adaptive nonlinear cell mass estimator yields excellent performance, i.e. long-term robustness and the elimination of state variable offset at steady state.

In recent years, a model-independent approach to on-line bioprocess identification has been taken. This approach, which is based on "pattern-recognition" theories, uses historical performance data to establish standard process patterns that contain all information necessary for effective process classification and process variable identification (Saner and Stephanopoulos, 1992; Bakshi and Stephanopoulos, 1994; Gollmer and Posten, 1995). Stephanopoulos et al. (1995) presented a pattern recognition methodology for fermentation data mining. This method consists of three components: a wavelet signal decomposition for effective data compression, a triangular representation of process signals aiming at the extraction of key process trends, and a decision tree for establishing the most direct relationship between batch run outcome, process variables, and trends. By incorporating wavelet decomposition and trend analysis information from historical fermentation process data into decision tree searches, distinguishing features in the signals are exposed. Following the rules generated by the decision tree, fermentation process yields can be optimized in future runs, and at the same time the most important process variables are identified.

2.3 Efficient Control Strategies for Continuous Bioreactors

The "control" of biotechnological plants is a complex problem. From the biological point of view, different substrates can be used and these are converted by a variety of microorganisms under anaerobic or aerobic conditions to the desired products. From the engineering or technological point of view, there are different reactor types: stirred tank, tube, tower, cascaded reactors, etc., and different modes of reactor operation: batch, fed-batch, continuous, which all pose different problems in terms of control system structure (Chattaway et al., 1991).

The primary objectives of control systems are to provide quality assurance and economic incentives. Slow dynamics associated with microbial bioreactors can result in off-specification products over a significant period for continuous operation. Unforeseen disturbances in a continuous bioreactor can result in a failure (washout) of the bioreactor, requiring new start-ups. Failures or off-specification products can have catastrophic economic consequences. Therefore, there is a strong economic incentive for proper control and optimization of bioreactors.

The purpose of control is to manipulate the control variables to: (1) maintain

the desired outputs at a constant desired value (a set-point problem), (2) stabilize unstable or potentially unstable processes such as continuous processes (a stabilization problem), and (3) optimize the performance as defined by measures such as yield, productivity, or profit (an optimization problem). These objectives are to be achieved under various constraints such as safety, environmental regulations, limited resources, and operational constrains.

In recent years, a significant number of efficient control strategies for bioreactor systems have been proposed, and these techniques have also been reviewed and critically evaluated (Johnson, 1987; Chattaway et al., 1991; Lim and Lee, 1991; Shimizu, 1993). Considerable emphasis has been placed on the control of fed-batch fermenters primarily because of their prevalence in industry traditionally. However, when the production of biomass or product is to be optimized, continuous operation is desirable. From an academic viewpoint, the continuous bioreactor is suitable for studying both transient and steady state behavior, and is of interest for investigations of microorganism physiology, as the individual process variables can be altered independently. In the following, a survey is presented for the variety of control strategies applied to continuous bioprocesses. There are essentially following different approaches that can be adopted in control strategy design:

- Conventional continuous bioreactor control configurations
- Adaptive control of continuous bioreactor
- Optimizing control of continuous bioreactor
- Nonlinear control of continuous bioreactor

2.3.1 Conventional continuous bioreactor control configurations

The conventional continuous bioreactor control configurations, which have appeared in literature (Agrawal and Lim, 1984), have been given special names. They include turbidostats, nutristats, pH-auxostats, and those based on the oxygen absorption rate, the oxygen uptake rate, and the carbon dioxide evolution rate. In these control configurations, a representative variable, which characterizes the steady state balanced growth of microorganisms in a continuous bioreactor, is controlled by manipulating the flow of nutrient medium to a culture vessel. A feedback PID controller is commonly used. We consider here the two

most common control configurations: a turbidostat where cell concentration is controlled in the form of turbidity, and a nutristat where substrate concentration is controlled.

An early publication, which discusses extensively the automatic control of continuous bioreactor, is that of Edwards et al. (1972) who analyzed and compared these two configurations for a continuous culture subject to substrate-inhibition kinetics. The uncontrolled continuous cultures of microorganisms grown on inhibitory levels of substrates are known to be unstable and liable to washout. They concluded that either of these two simple control configurations would eliminate the instability of such cultures for the typical range of operating conditions, and found that appreciable improvements in process regulation could be attained with the nutristat configuration.

Agrawal and Lim (1984) evaluated the conventional turbidostat, nutristat and other control configurations by using only steady state information. The feasibility of these control configurations was tested by checking the local controllability and/or stability criteria, while the practical effectiveness was evaluated by analyzing the steady state gains. They concluded that both the conventional turbidostats and nutristats are not effective at substrate limited growth conditions because of very low steady state gain and difficulty of accurately measuring the very low substrate concentration. They then proposed a modified turbidostat with two feed streams, a sterile water stream and a stream containing the concentrated amounts of the limiting substrate and other nutrients. The flow rate of the water stream was used as the manipulated variable, whereas the flow rate of the concentrated stream was kept constant. They found that this modified turbidostat is superior to the conventional turbidostat as it can operate under all growth conditions, and this new control configuration also provides the possibility of multivariable control of continuous bioreactors. Later, the efficacy of the modified turbidostat configuration was experimentally examined by Agrawal (1987). The experimental results illustrated the feasibility of the control configuration for effective startup and stabilization of a continuous bioreactor under the influence of a load disturbance.

Menawat and Balachander (1991) proposed an alternate control configuration where the feed substrate concentration was considered as the alternate manipulated variable. They claimed that the control performance for control of either cell concentration or substrate concentration is improved, compared to the conventional control configurations using the feed flow rate as the manipulated variable

which resulted in a sluggish and non-robust response. The control performance considered in their study was defined in terms of the ability to meet the control objective with minimum input effort.

The obvious advantages of the simple single-loop feedback control are its simplicity and low cost, and in most situations it is adequate to use. However, there are situations in which simple single-feedback control is inadequate, and more advanced control is required. More advanced control can improve the performance of control over that achievable by simple feedback control and has been shown to be successful as will be discussed in following sections.

2.3.2 Adaptive control of continuous bioreactor

In the above control configurations, a fixed structure PID controller is used. Unfortunately, the time-varying properties of fermentation processes can make it difficult to set "optimum" values for standard PID controllers in all but the simple standard bioreactor environment control loops (e.g. pH, Level, temperature control). Thus a major problem in closed loop fermentation control is the tuning of the controller parameters. It is for this reason that adaptive controllers, the parameters of which can be estimated (identified) and automatically varied on-line to compensate for variations in the fermentation process characteristics, have been studied quite extensively and here we briefly highlight some significant controllers.

An example of multivariable adaptive control to the continuous yeast fermentation was provided by the simulation studies of Andersen and Jørgensen (1988). The multivariable adaptive control method consisted of an optimal linear quadratic (LQ) controller combined with identification of a linear ARMAX-model. Two controlled (biomass and ethanol) and two manipulated variables (dilution rate and concentration of glucose in the feed) were used. The multivariable adaptive controller was compared with two decoupled PI controllers for set-point tracking and disturbance rejection. The simulation results indicated, not surprisingly, that the multivariable adaptive controller generally has better performance than the two PI controllers. The most significant difference was in the control effort, that is, the use of dilution rate is much less for the multivariable adaptive controller than for the PI controller.

Later, Jørgensen et al. (1992) presented experimental results for adaptive control of continuous yeast fermentation near the critical dilution rate (in order

to obtain the maximal productivity of biomass and to avoid significant ethanol production). The adaptive LQ controller was used to control the ethanol concentration by manipulating the feed flow rate. A sequence of set-point changes was carried out in order to investigate the process behavior around the critical dilution rate. The adaptive LQ controller performed well experimentally in that it provided good regulatory and set-point tracking performance around the critical dilution rate.

In the simulation study of the continuous yeast fermentation pilot plant, Andersen et al. (1991) considered an alternative approach of robust controller design, and the performance of an Internal Model Controller (IMC) for disturbance rejection and set point tracking was compared with the LQ controller and PI controller. Similar performances were obtained for these three controllers, which were all designed on the basis of the experimentally identified model. However, for disturbance rejection the IMC and PI controllers were observed to result in a more sluggish response. The study indicated that improved PI controller performance could be obtained by using on-line model-based tuning.

Vigié et al. (1990) presented theoretical and experimental studies concerning the application of adaptive predictive control approach to the control of effluent substrate concentration in a continuous multistage bioreactor fermenting glucose by yeast.

Samaan et al. (1990) have experimentally studied the application of robust performance-oriented adaptive control algorithm to a continuous alcohol fermentation.

2.3.3 Optimizing control of continuous bioreactor

The high costs associated with many bioprocesses make optimization of bioreactor performance very desirable. Due to the complexity of microbial cultures and changes in culture conditions, feed conditions and environmental conditions, an adaptive feature is required in the optimization of bioreactors. Obviously the objective of adaptive optimizing control for continuous bioreactors is to operate the continuous bioreactors at steady state with optimum productivity (or some other performance objective) in accordance with enzymatic deactivation and environmental changes.

Rolf and Lim (1984; 1985) have developed an adaptive optimization algorithm based on dynamic model identification and applied it to maximize the cellular

productivity of a continuous yeast fermentation by manipulating the dilution rate. In this method, a simple dynamic linear input-output model is used and its parameters are estimated by using the recursive least-square method from transient data to track the process on-line. The gradient optimization algorithm combined with the steady state portion of the model is then used in determining the necessary control action to improve the performance of the continuous bioreactor. Both simulation studies and experimental results on the continuous yeast fermentation process showed that the adaptive optimization method requires no detailed model, it is easy to implement, it converges quickly, it adapts to changes in the process, and it is stable even when operational difficulties are encountered.

The stability and tracking ability of the optimization algorithm strongly depends on the value of the forgetting factor in the recursive least-square routine. Therefore two modified versions of the above mentioned optimization algorithm were considered, in which the forgetting factor is adjusted on-line according to the system requirements instead of to be constant value. Chang et al. (1988) developed a modified optimization algorithm with a bilevel forgetting factor (BFF), i.e. the forgetting factor is placed either at a maximum level or a minimum value according to the magnitude of the error between the measured and the estimated values of the state variables. The modified optimization algorithm by Semones and Lim (1989) is based on a variable forgetting factor (VFF) method, i.e. adaptive tuning of forgetting factor. The simulation results (Chang et al., 1988) indicated that when applied to maximize the cellular productivity of a continuous yeast fermentation by manipulating the dilution rate, the optimization algorithm with BFF yields better performance than those with constant forgetting factor or VVF. The performance was evaluated in terms of optimization speed and accuracy, adaptability, and long-term operational stability, the last issue is critical in the operation of continuous bioreactors.

Chang and Lim (1989) also extended the modified optimization algorithm with a bilevel forgetting factor to multivariable adaptive optimization of continuous bioreactors. Specially, the cellular productivity of a continuous yeast fermentation was maximized by manipulating both dilution rate and temperature. The simulation and experimental results showed that the algorithm was able to stably maintain the process around the optimum point for an extended period of time. The algorithm performed well in terms of optimization speed, adaptability to extreme external disturbance, and reoptimization capability.

Chang and Lim (1990) used a fast responding and readily on-line measurable

variable, the carbon dioxide evolution rate (CER), to replace the cell concentration in maximization of the cellular productivity of a continuous yeast fermentation. The cell concentration had been the first choice in their previous studies, however the optimization algorithms based on the cell concentration measurements is relatively sluggish since the cell concentration is slow in response to changes in the dilution rate. Simulation and experimental studies showed that the proposed fast inferential adaptive optimization algorithm based on the CER measurement was two or three time faster than the algorithm based on cell concentration measurements.

Shi et al. (1989) proposed several adaptive on-line optimizing control strategies and tested them by computer simulation. The control task was divided into two: the upper layer searches for the optimal set point and passes it to the lower layer, of which the task is to make the process output follow the optimal set point as quickly as possible. It was shown to be effective for the upper layer to express the objective function as a polynomial of the measurement variable and this can be used to find the optimal set point. It was also shown to be effective to use the discrete type self-tuning PID controller and the optimal controller compensated for the interaction between the control loops in the lower layer. Application was made to continuous lactic acid production with a cell recycle system and the continuous baker's yeast cultivation.

2.3.4 Nonlinear control of continuous bioreactor

The adaptive control approaches discussed above primarily utilize a linear time series model of the fermentation. Since the underlying properties of the fermentation process are non-linear, improved control performance could be expected by explicitly taking into account known process non-linearities in the controller design. Fermenter nonlinearities can be addressed explicitly using "Exact Linearization Techniques" based on concepts from "Differential Geometry" (Isidori, 1989; Henson and Seborg, 1991). Using nonlinear coordinate transformations and nonlinear state feedback, the original nonlinear model can often be transformed into an equivalent linear model and the well established linear controller design techniques can then be employed. The model can be linearized in either an input-output sense or a state-space sense. As summarized by Bastin and Dochain (1990), the difference between this exact linearizing control technique and conventional control technique lies in the way that linearization is introduced

in the problem. In a conventional approach, one first calculates a linearized approximation of the model and a *linear controller* is designed for this approximate model. But the *closed loop* remains *nonlinear* and is guaranteed to be stabilized only locally but not over a wide range of operating points. In the exact linearizing control approach a *nonlinear controller* is precisely designed to achieve a *linear closed loop* which is unconditionally stable whatever the operating point.

Bastin and Dochain (1990) have developed adaptive nonlinear controllers based on the input-output linearization to cope with bioprocess nonlinearity and parameter uncertainty. A successful application of an adaptive nonlinear controller to a pilot-scale anaerobic digester was presented by Renard et al. (1988). A simple nonlinear mathematical model of the fermentation process without requiring any analytical expression for the specific growth rate was the basis for the derivation of the control law. By introducing nonlinearities of the system into the control scheme, a nonlinear feedback control was used in order to obtain a first-order linear kinetics for the closed-loop system. The introduction of the on-line estimate of the controller parameter gives the adaptive feature to the control law. Ramseier et al. (1991) described studies of SISO and MIMO adaptive versions of nonlinear Generic Model Control (GMC). An *a priori* nonlinear representation of process knowledge is combined with a simple adaptive scheme in order to deal with the bioprocess nonlinearities. Experiments with a continuous yeast fermentation system demonstrate for the SISO case the superior performance of the adaptive GMC algorithm over that using a conventional PI controller. In most of these studies, the productivity of the fermenter was *indirectly* controlled by regulating either the cell, substrate, or product concentrations at a specified value. If the fermenter exhibits significant time-varying behavior, this approach may result in suboptimal performance.

Henson and Seborg (1992) developed nonlinear controllers that provide *direct* productivity control. The control objective is to regulate the fermenter near the optimum productivity. The resulting controllers are compared theoretically and *via* simulation. It was shown that state-space linearization techniques are not appropriate for this class of fermenters. Exact input-output linearizing control employing the dilution rate as the manipulated input is shown to provide excellent regulatory behavior. Conversely, input-output linearization with the feed substrate concentration as the manipulated input is problematic, and they proposed a modified input-output linearization based on holding the feed substrate concentration constant near the optimum that results in satisfactory control.

Pröll and Karim (1994) have applied two differential geometry feedback control design approaches to nonlinear multivariable control of a continuous bioreactor. They concluded from simulation results that input/output linearization, with the ability to implement independent and decoupled control loops, gives more satisfactory results than the exact linearization approach. Exact linearization via state feedback shows a high degree of coupling on the controlled variables and large changes on the manipulated variables.

2.4 Concluding Remarks

In recent years computer technology has had a significant impact on biotechnological processes through the implementation of advanced control and optimization strategies. This area can provide significant improvement in product quality, yield and productivity. The extent of computer application depends on the development of better on-line instrumentation that can monitor cellular physiology, as well as on reliable mathematical models that can describe various cellular dynamics.

Presently, the effective on-line sensors are still limited for use in many cases, therefore on-line estimation techniques have been developed and have played a very important role in control and optimization of bioprocesses, as exemplified by these references cited in Section 2.2.

The review given in Section 2.3 reveals that there have been a significant number of studies on adaptive control and nonlinear control approaches to continuous bioprocesses. Adaptive control techniques form an area of active and developing research. However, while academic workers reports successes in specific applications, experience in bioprocess industry applications is sparse. Few adaptive controllers have found their way into major production processes.

Although the "nonlinear control" strategy can cope with the nonlinearity associated with bioprocesses, this method relies heavily on the accuracy of the mathematical models. The theoretical part has been well investigated with a number of successful simulation and pilot plant studies reported, but the practical application in industrial scale is quite limited so far.

From the practical application point of view, the "optimizing control" is quite important. The success of optimizing the performance of biotechnical system depends upon the availability of a representative mathematical model of the process and the selection of an appropriate optimization routine.

Finally, it must be stressed that control consideration should be taken into account early in the design stage for a biotechnical process. This will assure not only controllability of the bioprocess, but also optimal overall performance of the entire system. The important thing in considering the control problem is not simply to apply sophisticated control theory, but to consider it with deep understanding of the dynamics of physiological state changes and incorporating as more as possible biological knowledge.

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Chapter 3

A Comparison of Various Control Configurations for Continuous Bioreactors

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Abstract

Five control configurations for continuous bioreactors are examined based on the controllability analysis with respect to disturbance rejection. A controller-independent controllability measure, the partial disturbance gain, is used for the evaluation. In particular, we have generalized the partial disturbance gain, which was first proposed by Skogestad and Wolff (1992) confined to the case that only one output is uncontrolled with other loops closed, to the common case with more outputs uncontrolled. At substrate-limited growth conditions, the turbidostat using the feed substrate concentration as the manipulated variable is the best control configuration. The conventional turbidostat should be avoided to use. When the cell growth is not substrate limited, all these control configurations are effective except the nutristat using feed substrate concentration as manipulated variable which is not feasible no matter whether the growth is substrate limited or not.

3.1 Introduction

Bioreactor control has become an active area of research in recent years. Much emphasis has been placed on the control of fed-batch bioreactors because of their prevalence in industry traditionally. However, when production of cell mass or product is to be optimized, continuous operation is desirable.

Unforeseen disturbances in a bioreactor may result in a failure in the operation of the reactor, such as *washout*, and require a new start-up procedure. Therefore, there is a strong economic incentive to develop efficient control strategies that would enable rapid start-up and stabilization of steady states in continuous bioreactors subject to disturbances.

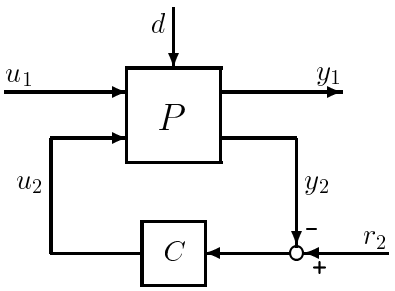
Problem formulation. As already noted the primary objective of a continuous bioreactor control system is usually to avoid that the reaction stops due to washout. This may be done by closing one feedback loop and controlling the cell mass or substrate concentrations (X or S [g/l]). In addition, in order to optimize the reaction and maintain the quality of the product, one may as secondary control objective want to keep both X and S at some desired values. To do this two degrees of freedom are available, namely the total feed flow rate, F [l/h] (which is usually normalized with the reactor volume to get the dilution rate, $D = F/V$ [h^{-1}]) and the feed substrate concentration, S_f [g/l]. We then have that the candidate inputs and outputs are

$$u = \begin{bmatrix} F \\ S_f \end{bmatrix}, \quad y = \begin{bmatrix} X \\ S \end{bmatrix}$$

One obvious solution is to implement a 2×2 control configuration. However, this is expensive and probably not necessary, because microorganisms have *intracellular* regulatory mechanisms and thus there exist strong interactions between the two outputs. In this paper the main consideration is therefore on whether one may achieve acceptable control performances of both outputs by controlling only one of them. This gives rise to four possible 1×1 control configurations. Actually, there are more possibilities for selecting inputs. For example, to adjust the total feed flow rate (F), we can adjust F directly, or as in the modified turbidostat with two feed streams proposed by Agrawal and Lim (1984), we can use either the flow rate of the concentrated substrate stream (F_S) or the flow rate of the sterile water stream (F_w).

This is actually a quite general control strategy problem where one is faced with a "partial control problem", that is, to use a subset of the available inputs

to control a subset of the outputs. Define $y = [y_1 \quad y_2]^T$ and $u = [u_1 \quad u_2]^T$ as shown in Figure 3.1.



Plant:
$P = (G \quad G_d)$

Figure 3.1: Simplified partial control system

The problem is to select the controlled outputs (y_2), and the manipulated inputs (u_2). Issues are:

1. Use of u_2 to control y_2 should yield satisfactory control performance (u_2 should have a "large" effect on y_2 to avoid input constraints; delays and RHP-zeros from u_2 to y_2 should not conflict with the desired bandwidth needed for disturbance rejection and set-point tracking).

2. With these control loops closed (i.e. with y_2 approximately constant) and with the unused inputs u_1 constant, the uncontrolled outputs y_1 should be relatively insensitive to disturbances d.

Issue 1 is a conventional feedback control problem where dynamic issues generally are most important, while steady-state considerations are often more important for issue 2. To evaluate both these issues the controllability analysis of the alternative structures needs to be performed. A useful tool when considering issue 2 is the partial disturbance gain proposed by Skogestad and Wolff (1992) which is the effect of a disturbance on the uncontrolled output when the controlled outputs are kept constant. A more general definition of the partial disturbance gain will be given in Section 3.2.

Such strategy decisions arise frequently in process control. One extensively studied problem is the control configuration selection for distillation columns (e.g. Skogestad and Morari, 1987). For distillation columns it is well known that some configurations have better "built-in" disturbance rejection ability than others, i.e. the sensitivity of the uncontrolled outputs with respect to disturbances is less.

The issue of disturbances has not been widely discussed in the literature on the controllability analysis for bioreactors. The objective of this paper is then to make a comparison of various control configurations for continuous bioreactors based on the controllability analysis with respect to disturbance rejection.

Control Configurations Studied. In this paper five control configurations are examined:

1. Conventional turbidostat ($D \rightarrow X$ – configuration). Dilution rate (D) is used to control the cell mass concentration (X).

2. Conventional nutristat ($D \rightarrow S$ – configuration). D is used to control the substrate concentration (S).

3. Concentration turbidostat ($S_f \rightarrow X$ – configuration). Feed substrate concentration (S_f) is used to control X (Menawat and Balachander, 1991).

4. Concentration nutristat ($S_f \rightarrow S$ – configuration). S_f is used to control S (Menawat and Balachander, 1991).

5. Modified turbidostat ($D_w \rightarrow X$ – configuration). Dilution rate of the sterile water stream (D_w) is used to control X (Agrawal and Lim, 1984).

Previous work. The two most common control configurations are the turbidostat and nutristat with the dilution rate D as a manipulated input. In a turbidostat the cell mass concentration X is controlled (in practice, the optical density is used to infer X). In a nutristat the residual substrate concentration S is controlled. Edwards et al. (1972) analyzed and compared these two configurations for a bioreactor with substrate inhibition kinetics and they concluded that the nutristat is superior to the turbidostat in many applications. Agrawal and Lim (1984) evaluated the turbidostat, nutristat and other control configurations based on local controllability, local stability, input multiplicity and steady state gains. They concluded that the conventional turbidostat and nutristat are feasible only at those conditions where the cell growth is not substrate limited. They then proposed a modified turbidostat with two feed streams, a sterile water stream and a stream containing the concentrated amounts of the limiting substrate and other nutrients. The dilution rate of the sterile water stream (D_w) is used as the manipulated variable with the concentrated substrate stream of constant flow (D_S). They found this modified turbidostat to be superior to the conventional

turbidostat in that it can operate under all growth conditions. Menawat and Balachander (1991) proposed to use the feed substrate concentration S_f as the manipulated variable and claimed that this control configuration improves the control performance for control of either X or S compared to using the dilution rate as the manipulated variable.

3.2 Controllability Measure

In this paper simple frequency-dependent tools are used to study the inherent control characteristics. In particular, the open-loop disturbance gain G_d (for selecting the "controlled" pairings) and the partial disturbance gain P_d (for selecting the "uncontrolled" pairings) are considered.

Tools. Consider a linear model of the form

$$y(s) = G(s)u(s) + G_d(s)d(s) \tag{3.1}$$

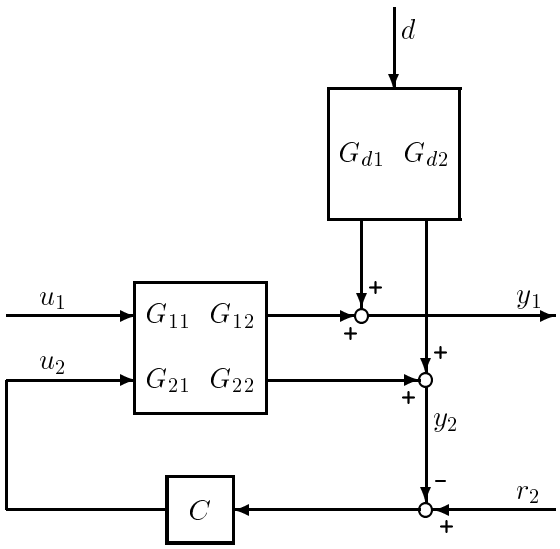


Figure 3.2: Block diagram of a partial control system

As shown in Figure 3.2, we further rearrange and partition the model $y = Gu + G_d d$ such that the first part of y contains the uncontrolled outputs (y_1), and the first part of u contains the unused inputs (u_1), whereas the remaining outputs y_2

are controlled using the remaining inputs u_2. We then have

$$y_1 = G_{11}u_1 + G_{12}u_2 + G_{d1}d \tag{3.2}$$

$$y_2 = G_{21}u_1 + G_{22}u_2 + G_{d2}d \tag{3.3}$$

The open-loop disturbance sensitivity for outputs y_1 and a disturbance d is

$$\left(\frac{\partial y_1}{\partial d}\right)_u = G_{d1} \tag{3.4}$$

The corresponding partial disturbance sensitivity for y_1 with outputs y_2 perfectly controlled by using u_2 is defined

$$P_{d1} \stackrel{\text{def}}{=} \left(\frac{\partial y_1}{\partial d}\right)_{\{y_2=0\} \text{ using } \{u_2\}} \tag{3.5}$$

After further derivations (see Appendix), we get the following analytical expression for calculating the partial disturbance gain,

$$\boxed{P_{d1} = G_{d1} - G_{12}G_{22}^{-1}G_{d2}} \tag{3.6}$$

The partial disturbance gain means the disturbance gain for a system under partial control. The advantage with the partial disturbance gain is that the partial disturbance gain only depends on the plant itself, i.e. the partial disturbance gain is controller-independent controllability measure. In particular, P_{d1} is a vector for a single disturbance d, and P_{d1} is a matrix for k simultaneous disturbances $d = [d_1 \cdots d_k]^T$. We use the induced 2-norm[†] of P_{d1}, $\|P_{d1}\|_{i2} = \bar{\sigma}(P_{d1})$ (e.g. Skogestad and Postlethwaite, 1996, p. 518), to evaluate the worst *overall* effect of *simultaneous* disturbances on uncontrolled outputs y_1. Note: $\|A\|_{i2}$ is equal to $\|A\|_2$ (Euclidean norm) when A is a vector, so for simplicity we use $\|A\|_{i2}$ throughout the paper both when A is a matrix or vector.

Summary of controllability analysis. The variables should first be scaled with respect to their allowed or expected range such that all variables are less than 1 in magnitude (norm). The objective of the controllability analysis in this paper

[†]From a scaling point of view, the induced infinity-norm (e.g. Skogestad and Postlethwaite, 1996, p. 518) may be preferable. However, in the thesis the *overall* effect of *simultaneous* disturbances is mostly concerned. We choose to use the induced 2-norm as it gives the average effect of all elements (disturbances), whereas the induced infinity-norm reflects the worse-case effect (the maximum element or maximum row sum).

is to find the best partial control configuration, that is, to select the controlled outputs (y_2) (and inputs u_2) such that the norm of P_{d1}, $\|P_{d1}\|_{i2}$ is small, at least we want $\|P_{d1}\|_{i2}$ smaller than $\|G_{d1}\|_{i2}$. The controllability analysis then consists of two main steps:

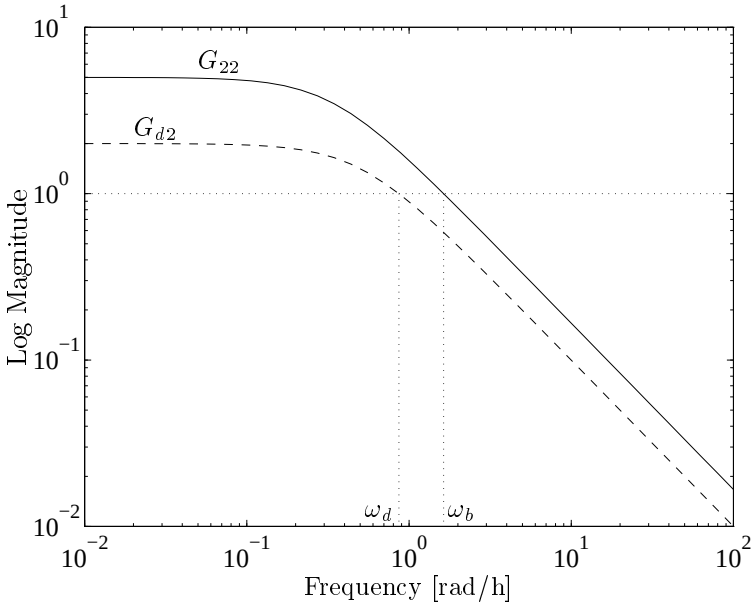


Figure 3.3: Selection of "controlled" subsystem

1. *Analysis of "controlled" subsystem with "controlled pairing"* $u_2 - y_2$ (see Figure 3.3)

   (a) To get the acceptable speed of response: Such pairings are preferred that G_{22} has no delays and RHP-zeros within the desired bandwidth range. The desired frequency bandwidth ω_b should be larger than the frequency range ω_d where for some disturbance d, $\|G_{d2}(j\omega)\|_{i2} > 1$, i.e. we want $\omega_b \geq \omega_d$.

   (b) To avoid input constraints: Find pairings where $\|G_{22}^{-1} G_{d2}\|_{i2} \leq 1$ so that $\|u_2\|_{i2} \leq 1$ (by setting $u_1 = 0$ in Eq. (3.28) in Appendix) within the desired bandwidth range even under the worst case where $\|d\|_{i2} = 1$.

2. *Analysis of "uncontrolled" subsystem with "uncontrolled pairing"* $u_1 - y_1$. To get the acceptable disturbance sensitivity for the uncontrolled outputs: Such pairings are preferred that the magnitude of the corresponding partial disturbance gain, $\|P_{d1}\|_{i2} \leq 1$ so that $\|y_1\|_{i2} \leq 1$ (by setting $u_1 = 0$ in Eq. (3.29) in Appendix) at all frequencies even under the worst case where $\|d\|_{i2} = 1$.

Therefore, to evaluate the feasibility of partial control one must for each choice $u_2 - y_2$, rearrange the system as in Eqs. (3.2)–(3.3) and calculate P_{d1} using Eq. (3.6).

3.3 Bioreactor Model

A schematic diagram of a continuous bioreactor is shown in Figure 3.4.

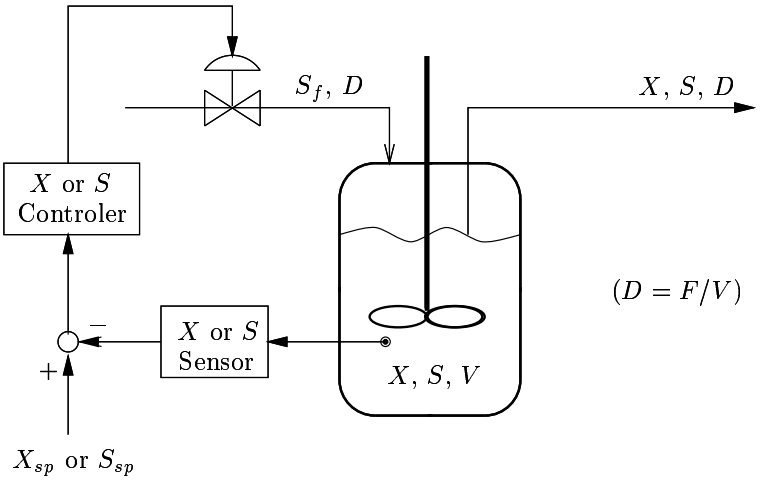


Figure 3.4: A schematic diagram of a continuous bioreactor

From a chemical engineering point of view it can be viewed as a continuous stirred tank reactor (CSTR) with well-mixed contents and constant volume. The dilution rate (D) and the feed substrate concentration (S_f) are available as manipulated inputs. The cell mass concentration (X) and substrate concentration S are the process state variables and we assume that X and S are available for controller design. Although this assumption is rarely satisfied in practice, these state variables can often be estimated from secondary variables such as oxygen consumption rate and carbon dioxide production rate. The most important overall reac-

tion for this study is the growth of the microorganism population on the substrate:

$$\text{substrate} \xrightarrow{\curvearrowleft} \text{cells} + \text{metabolic product}$$

From a biochemical point of view this reaction is auto-catalytic since the cell mass is a catalyst of its own production as indicated by the feedback arrow '$\curvearrowleft$' (there is no cell mass growth without initial cell mass added) but is not consumed by the growth reaction (Bastin and Dochain, 1990). In other words, the rate r [g cells/(h $\cdot$ l)] for formation of new cells increases with cell concentration itself. Usually, the formation rate r is assumed to be proportional to the concentration of cells (X), i.e. $r = \mu(S)X$. Here the specific growth rate (μ [h^{-1}]) depends on the substrate concentration (S), as given by Monod model which is most commonly used:

$$\mu(S) = \frac{\mu_m S}{K_s + S} \tag{3.7}$$

where μ_m and K_s represent the maximum specific growth rate and the saturation constant respectively. A variety of fermentations can be described by the following "unstructured"[‡] model which is the result of the material balances on the cell mass and the substrate in a constant-volume continuous bioreactor:

$$\boxed{\frac{dX}{dt} = \mu(S)X - DX} \tag{3.8}$$

$$\boxed{\frac{dS}{dt} = D(S_f - S) - \frac{\mu}{Y_{X/S}}X} \tag{3.9}$$

Here $Y_{X/S}$ is the yield coefficient [g cells/ g substrate] which is assumed constant in this paper.

3.3.1 Steady state behavior

From Eqs. (3.8) and (3.9), the steady state values of cell mass and substrate concentrations can be calculated[§]:

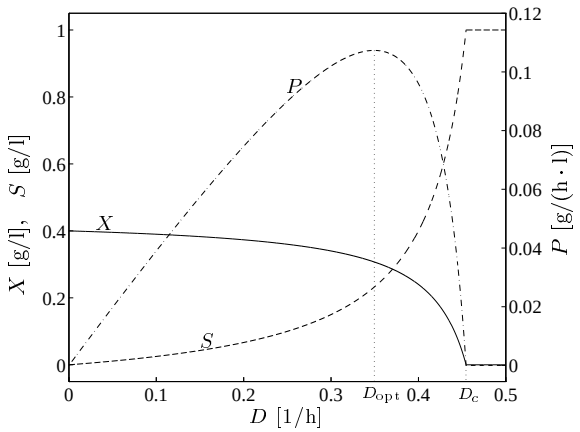
$$\frac{dX}{dt} = 0 \quad \Rightarrow \mu(\overline{S}) = \overline{D} \quad \Rightarrow \boxed{\overline{S} = \frac{K_s \overline{D}}{\mu_m - \overline{D}}} \tag{3.10}$$

$$\frac{dS}{dt} = 0 \qquad\qquad \Rightarrow \boxed{\overline{X} = Y_{X/S}\,(\overline{S_f} - \overline{S})} \tag{3.11}$$

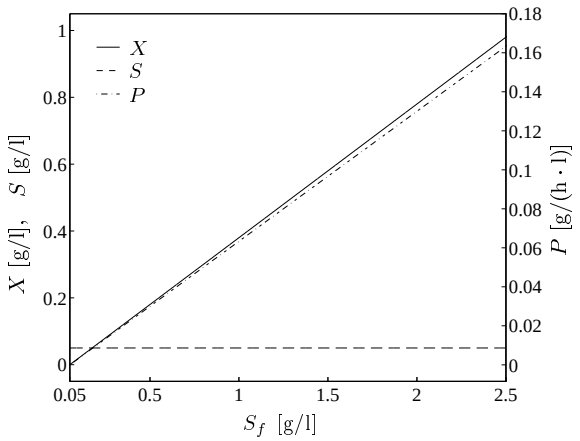
[‡]The term "unstructured" designates that the fermentation is assumed to be dominated by a single, homogeneously growing microorganism.

[§]For other kinetic schemes, like the substrate inhibition kinetics (Edwards et al., 1972) multiple steady states may exist.

Here the overbar ($^-$) denotes steady-state values. The steady-state relationship is shown graphically in Figure 3.5.



(a) Effect of D with $S_f = 1$ g/l



(b) Effect of S_f with D=0.17 h^{-1}

Figure 3.5: Steady state behaviors of X, S and P as a function of (a) D and of (b) S_f. Values of μ_m, K_s and $Y_{X/S}$ from Table 3.1.

As can be seen from Eq. (3.10), the steady-state substrate concentration ($\overline{S}$) is independent of the feed concentration ($\overline{S_f}$). This unusual feature is due to the auto-catalytic reaction. Thus $\overline{S}$ depends only on the dilution rate ($\overline{D}$). On the other hand, $\overline{X}$ depends also on $\overline{S_f}$ as given by Eq. (3.11). Another phenomenon

caused by the auto-catalytic reaction is the possibility for washout. Mathematically, washout corresponds to the case when the above steady-state equations have no physical solution. Specifically, $\overline{X}$ in Eq. (3.11) must be positive, that is, we must require that $\overline{S_f} > \overline{S}$. Inserting this requirement into Eq. (3.10) we find that dilution rate $\overline{D}$ must be less than a critical value D_c which depends on $\overline{S_f}$:

$$D_c = \frac{\mu_m \overline{S_f}}{K_s + \overline{S_f}} = \mu(\overline{S_f}) \tag{3.12}$$

Physically, at high dilution rates $\overline{D} > D_c$ (as shown in Figure 3.5(a)), the cells can not grow fast enough to keep up with its dilution, and the culture is washed out of the reactor. Similarly, at the other extreme, at very low but nonzero dilution rates, a large fraction of the cells may die from starvation and then the reaction stops since the limiting substrate is not being added enough to permit maintenance of cell metabolism, but this effect is not reflected by our simple model since no maintenance term is included.

For many continuous bioreactors the objective is to maximize the cell productivity $P = DX$ [g cells/(h·l)]. As shown in Figure 3.5, the productivity increases when S_f increases, but for changes in D it has a maximum value (set $dP/dD = 0$) corresponding to:

$$D_{\text{opt}} = \mu_m \left(1 - \sqrt{\frac{K_s}{K_s + \overline{S_f}}}\right) \tag{3.13}$$

The nominal model parameter values and steady state data for two operating points are given in Table 3.1. Here operating point No. I is the one studied by Menawat and Balachander (1991) and operating point No. II corresponds to the maximum productivity.

Table 3.1: Steady State Data

Operating Point	$\overline{D}$ [h^{-1}]	$\overline{S_f}$ [g/l]	$\overline{X}$ [g/l]	$\overline{S}$ [g/l]	$\overline{P} = \overline{D}\,\overline{X}$ [g/(h · l)]
No. I	0.17	1.0	0.38	0.05	0.065
No. II	0.35	1.0	0.31	0.23	0.109
washout	> 0.46	1.0	0	1.00	0
Nominal model parameter	μ_m [h^{-1}] 0.5		K_s [g/l] 0.1		$Y_{X/S}$ [g/g] 0.4

3.3.2 Linearized model

Linearizing Eqs. (3.8) and (3.9) around a nontrivial steady state yields the transfer function model

$$y(s) = G(s)u(s) + G_d(s)d(s) \tag{3.14}$$

$$y = \begin{bmatrix} \Delta X \\ \Delta S \end{bmatrix}, \quad u = \begin{bmatrix} \Delta D \\ \Delta S_f \end{bmatrix} \left(\text{or } u = \begin{bmatrix} \Delta D_w \\ \Delta D_S \end{bmatrix} \right) \tag{3.15}$$

$$d = \begin{bmatrix} \Delta\mu_m & \Delta K_s & \Delta Y_{X/S} & \Delta D_d & \Delta S_{fd} \end{bmatrix}^T \left(\text{or } d = \begin{bmatrix} \Delta\mu_m & \Delta K_s & \Delta Y_{X/S} & \Delta D_{wd} & \Delta S_{fd} & \Delta D_{Sd} \end{bmatrix}^T \right) \tag{3.16}$$

where the outputs (states) y, inputs u and disturbances d represent deviations from the steady state. In addition to possible disturbances in the manipulated inputs D and S_f, we have included disturbances in the model parameters μ_m, K_s and $Y_{X/S}$ which may stem from variations in the environment conditions such as temperature, pH, aeration rate etc. The input-output transfer function matrix from $\begin{bmatrix} \Delta D \\ \Delta S_f \end{bmatrix}$ to $\begin{bmatrix} \Delta X \\ \Delta S \end{bmatrix}$ is

$$G(s) = \begin{bmatrix} \frac{-\overline{X}}{s+a} & \frac{\overline{D}\,\overline{X}}{(s+a)(s+\overline{D})}\overline{\frac{d\mu}{dS}} \\ \frac{\overline{X}}{(s+a)Y_{X/S}} & \frac{s\overline{D}}{(s+a)(s+\overline{D})} \end{bmatrix} \tag{3.17}$$

where $a = \frac{\overline{X}}{Y_{X/S}}\overline{\frac{d\mu}{dS}}$.

Remarks about the model:

1. The model is stable at all non-trivial steady-states. At washout conditions $\overline{X} \to 0$ and the pole $a \to 0$, so the linearized model becomes unstable.

2. The steady-state gain matrix is obtained by setting $s = 0$. As expected S_f has no steady-state effect on S and therefore should not be used to control S.

3. Except for this zero gain between S_f and S, neither the transfer function nor any of its elements contains RHP-zeros. However, measurement delays in obtaining X or S have not been included which will limit the achievable performance.

4. Since the yield coefficient $Y_{X/S}$ is assumed constant, one of the eigenvalues is fixed at $-\overline{D}$ irrespective of the reaction, and it can be easily shown that there is a combined state, e.g. $Z = X + Y_{X/S}\,S$, representing a reaction invariant. We have:

$$\frac{dZ}{dt} = \frac{dX}{dt} + Y_{X/S}\frac{dS}{dt} \tag{3.18}$$

and substituting Eqs. (3.8) and (3.9) into Eq. (3.18) then yields:

$$\frac{dZ}{dt} = -D(Z - Y_{X/S}\, S_f) \tag{3.19}$$

This applies irrespective of the reaction kinetics $\mu(S)$, so Z is indeed a reaction invariant.

5. From the transfer function matrix the effect of D is first order and does not affect the combined state Z. Thus, the system is not "*state* controllable" with D as an input. Physically, if D is used to control X (or S) the uncontrollable combined state Z will "drift away" by itself unaffected by the feedback using D because the steady state value $\overline{Z} = Y_{X/S}\, \overline{S_f}$ is independent of D. Menawat and Balachander (1991) indicated that this may be a problem. However, as pointed out by Agrawal and Lim (1984) it may not have any practical significance since the uncontrollable system is *stable*, and it is possible to design a successful control system in which one state (output) X or S is controlled and any control problems associated with it will appear in the controllability analysis of the uncontrolled output.

3.4 Controllability Study

3.4.1 Scaling of variables

The scaled transfer matrices are derived by scaling all variables with respect to their maximum allowed changes.

$$G' = \begin{bmatrix} \overline{\Delta X} & 0 \\ 0 & \overline{\Delta S} \end{bmatrix}^{-1} G \begin{bmatrix} \overline{\Delta D} & 0 \\ 0 & \overline{\Delta S_f} \end{bmatrix} \tag{3.20}$$

$$G'_d = \begin{bmatrix} \overline{\Delta X} & \\ & \overline{\Delta S} \end{bmatrix}^{-1} G_d \begin{bmatrix} \overline{\Delta \mu_m} & & & \\ & \overline{\Delta K_s} & & \\ & & \overline{\Delta Y_{X/S}} & \\ & & & \overline{\Delta D_d} \\ & & & & \overline{\Delta S_{fd}} \end{bmatrix} \tag{3.21}$$

(in the following the prime ($'$) used to denote the scaled matrices is omitted to simplify notation). The allowed maximum output changes are

$$\overline{\Delta X} = 10\%\, \overline{X}; \quad \overline{\Delta S} = 20\%\, \overline{S}$$

The allowed maximum input changes are

$$\overline{\Delta D} = 30\%\,\overline{D}; \quad \overline{\Delta S_f} = 35\%\,\overline{S_f}$$

The expected maximum disturbance changes are

$$\overline{\Delta \mu_m} = 10\%\,\overline{\mu_m}; \quad \overline{\Delta K_s} = 20\%\,\overline{K_s}; \quad \overline{\Delta Y_{X/S}} = 25\%\,\overline{Y_{X/S}}$$

$$\overline{\Delta D_d} = 20\%\,\overline{\Delta D}; \quad \overline{\Delta S_{fd}} = 20\%\,\overline{\Delta S_f}$$

3.4.2 Controllability analysis results

Operating Point No. I. At this operating point the cell growth is substrate limited. The steady-state gain matrices in terms of scaled variables are

$$G(0) = \begin{bmatrix} -0.24 & 3.68 \\ 2.25 & 0 \end{bmatrix}$$

$$G_d(0) = \begin{bmatrix} 0.08 & -0.11 & 2.5 & -0.05 & 0.74 \\ -0.75 & 1 & 0 & 0.45 & 0 \end{bmatrix}$$

The time constants at this operating point are $1/a = 0.5$ h and $1/D = 5.9$ h. The corresponding frequency response plots are shown in Figure 3.6.

We now proceed with the controllability analysis in order to evaluate the five control configurations by following the controllability analysis procedures described in Section 3.2.

1. Controlled output.

a. Input constraints. The first requirement is that the open-loop gain, $|G_{ij}|$ for the "controlled pairing" should be larger than the disturbance gains $|G_{dik}|$ to avoid input constraints. We first note that the steady-state gain from S_f to S is zero which means that S_f can not be used to control S at low frequencies. The steady-state gain from D to X is only -0.24 whereas the gain for a disturbance in yield ($Y_{X/S}$) is about 2.5. This means that the control action in D needed to reject the largest disturbance in $Y_{X/S}$ (which corresponds to a change in $Y_{X/S}$ of 25%) is about $2.5/0.24 \approx 10$ times higher than what is allowed. The conventional turbidostat is therefore not recommended at operating point No. I where the cell growth is substrate limited. This is consistent with the conclusion of Agrawal and Lim (1984).

b. Bandwidth requirements. From the frequency-dependent plots of the elements in G_d, the bandwidth requirement to achieve acceptable rejection for disturbances in $Y_{X/S}$ (the worst disturbance) is 0.4 rad/h for X (response time of shorter than 2.5 h required) (see the dotted curve marked by G_{d13} in Figure 3.6(b)), and 3 rad/h for S (response time of shorter than 0.3 h required) (see the dotted curve marked by G_{d23} in Figure 3.6(c)). In practice, this means that it may be favorable to control X if very long measurement delays are expected.

2. Uncontrolled output.

The effect of disturbances on the uncontrolled output are given by the partial disturbance gain. The steady state values are listed for individual disturbances in Table 3.2, and the overall effect of simultaneous disturbances on the uncontrolled output, $\|P_d\|_{i2} = \bar{\sigma}(P_d)$, is shown as a function of frequency in Figure 3.6(d) where the superscripts i, j of $\bar{\sigma}(P_{dl}^{i,j})$ refer to the controlled output y_i and corresponding input u_j, and the subscript l denotes the uncontrolled output y_l.

From the steady state values in Table 3.2 and the frequency response of $\bar{\sigma}(P_d)$ in Figure 3.6(d), the best configuration is the $S_f \rightarrow X$ – configuration (see the dotted curve marked by $\bar{\sigma}(P_{d2}^{1,2})$ in Figure 3.6(d)). However, the $D \rightarrow S$ – configuration (see the dashed curve marked by $\bar{\sigma}(P_{d1}^{2,1})$ in Figure 3.6(d)) is better at higher frequencies. The $S_f \rightarrow S$ – configuration (see the solid curve marked by $\bar{\sigma}(P_{d1}^{2,2})$ in Figure 3.6(d)) is not feasible since the sensitivity to some disturbances is infinite, while the $D \rightarrow X$ – configuration (see the dashdot curve marked by $\bar{\sigma}(P_{d2}^{1,1})$ in Figure 3.6(d)) is unacceptable with large sensitivity to disturbances in $Y_{X/S}$ (24 times larger than acceptable) and in S_f (7 times larger than acceptable).

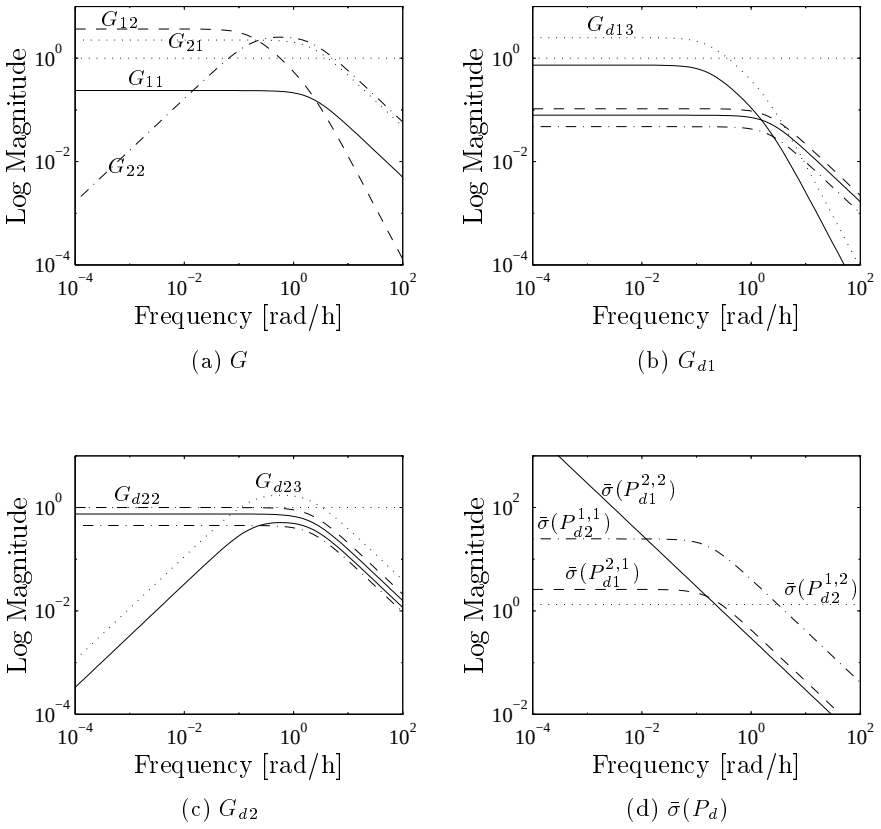
If the feed substrate concentration is difficult to manipulate, the $D_w \rightarrow X$ – configuration (modified turbidostat) proposed by Agrawal and Lim (1984) is quite effective. The steady-state gain from D_w to X (with D_S constant) is -1.70. The steady-state values of the partial disturbance gain giving the effect of various disturbances on the uncontrolled output S are

$$\begin{bmatrix} d_k \\ P_{d2k}^{1,1} \end{bmatrix} = \begin{bmatrix} \mu_m & K_s & Y_{X/S} & D_{wd} & S_{fd} & D_{Sd} \\ -0.7 & 0.93 & 1.66 & 0 & 0.49 & 0.42 \end{bmatrix}$$

which is almost acceptable. Here $P_{d2k}^{1,1}$ is the partial disturbance gain for the effect of the disturbance d_k on the uncontrolled output y_2 (S) with the output y_1 (X) controlled using the input u_1 (D_w). In particular, we note that the effects of disturbances in $Y_{X/S}$ and in S_f on the uncontrolled output S ($P_{d23}^{1,1} = 1.66$, $P_{d25}^{1,1} = 0.49$) are much less than for the conventional turbidostat ($P_{d23}^{1,1} = 23.75$,

Table 3.2: P_d at operating point No. I

Configuration		Disturbance				
No.	$u_j \rightarrow y_i$	μ_m	K_s	$Y_{X/S}$	D_d	S_{fd}
1	$D \rightarrow X$	0	0	23.75	0	7
2	$D \rightarrow S$	0	0	2.5	0	0.74
3	$S_f \rightarrow X$	-0.75	1	0	0.45	0
4	$S_f \rightarrow S$	∞	∞	0	∞	0



(a) G (b) G_{d1}

(c) G_{d2} (d) $\bar{\sigma}(P_d)$

Figure 3.6: Frequency responses at the operating point No. I

$P_{d25}^{1,1} = 7$). Thus, using the dilution rate of the sterile water stream (D_w) to control X gives much better control performance than using the total dilution rate (D) (conventional turbidostat). Actually, manipulating the dilution rate of the sterile water stream (D_w) is equivalent to manipulating the effective feed substrate concentration ($S_{ef} = \frac{D_S S_f}{D_w + D_S}$).

In conclusion, linear controllability analysis results show that the $S_f \rightarrow X$ – configuration is best at operating point No. I.

Operating Point No. II. This operating point corresponds to the maximum productivity where the cell growth is not substrate limited. We have

$$G(0) = \begin{bmatrix} -2.95 & 4.52 \\ 4.97 & 0 \end{bmatrix}$$

$$G_d(0) = \begin{bmatrix} 0.98 & -0.59 & 2.5 & -0.59 & 0.91 \\ -1.65 & 1 & 0 & 0.99 & 0 \end{bmatrix}$$

The time constants at this operating point are $1/a = 2.3$ h and $1/D = 2.9$ h. The corresponding frequency response plots are shown in Figure 3.7. We note immediately that the outputs are more sensitive to both inputs and disturbances than at operating point No. I. In particular, this is the case for changes in the dilution rate D where the gain for its effect on relative changes X has increased by more than a factor of 10. This means that tighter control is needed to reject disturbances in this case, and since the reactor is operating closer to washout, tighter control is needed also to avoid washout.

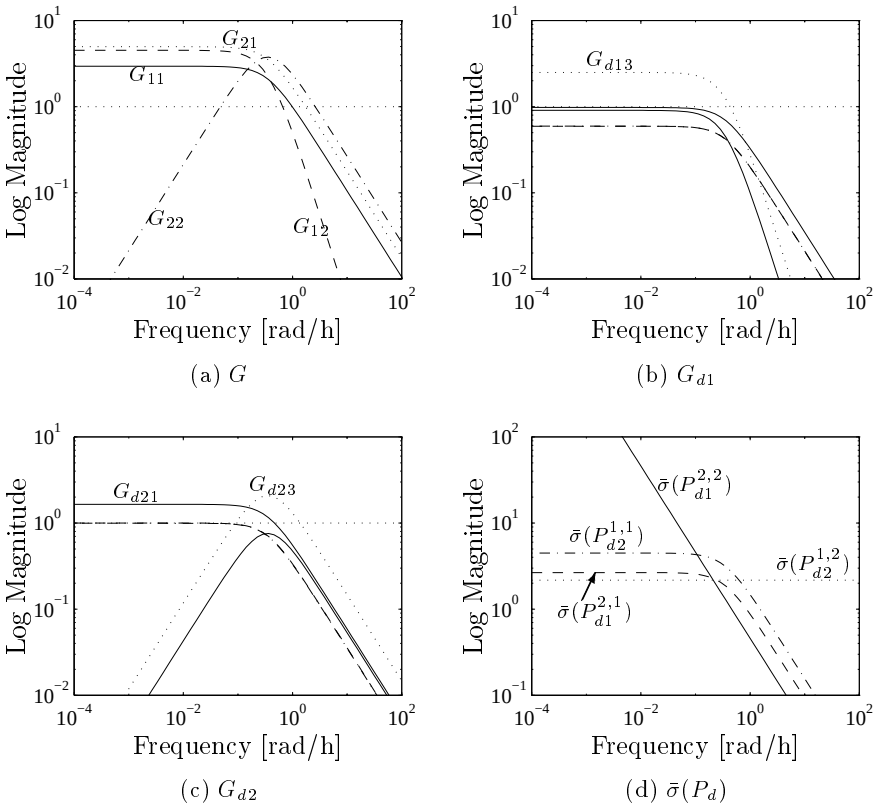
The steady-state values of the partial disturbance gain are listed in Table 3.3, and the overall effect of disturbances $\bar{\sigma}(P_d)$ is shown as a function of frequency in Figure 3.7(d).

From the steady-state values of the partial disturbance gain, and in particular the frequency response of $\bar{\sigma}(P_d)$ shown in Figure 3.7(d), the $D \rightarrow S$ – configuration (see the dashed curve marked by $\bar{\sigma}(P_{d1}^{2,1})$ in Figure 3.7(d)) is preferable; especially at high frequency range. However, at this operating point there is no big difference in terms of the controllability of these control configurations, except for the $S_f \rightarrow S$ – configuration (see the solid curve marked by $\bar{\sigma}(P_{d1}^{2,2})$ in Figure 3.7(d)) which is still not feasible. Also the $D \rightarrow X$ – configuration (see the dashdot curve marked by $\bar{\sigma}(P_{d2}^{1,1})$ in Figure 3.7(d)) may be efficient.

A more detailed discussion on the $D \rightarrow X$ – configuration (conventional turbidostat) is given in the following. For the case when the cell concentration X is

Table 3.3: P_d at operating point No. II

Configuration		Disturbance				
No.	$u_j \rightarrow y_i$	μ_m	K_s	$Y_{X/S}$	D_d	S_{fd}
1	$D \rightarrow X$	0	0	4.21	0	1.53
2	$D \rightarrow S$	0	0	2.5	0	0.91
3	$S_f \rightarrow X$	-1.65	1	0	0.99	0
4	$S_f \rightarrow S$	∞	∞	0	∞	0



(a) G (b) G_{d1}

(c) G_{d2} (d) $\bar{\sigma}(P_d)$

Figure 3.7: Frequency responses at the operation point No. II

perfectly controlled by the dilution rate D, we get

$$\Delta X = G_{11}\,\Delta D + G_{d1k} d_k = 0$$

$$|\Delta D| = \left|\frac{G_{d1k}}{G_{11}} d_k\right| \leq \left|\frac{G_{d1k}}{G_{11}}\right| < 1,\ \forall k = 1,\ ..., 5,$$

Then even under the maximum disturbance ($d_k = 1$), X can be perfectly controlled by D without encountering input constraint. For the uncontrolled substrate concentration S under a disturbance d_k, at the steady state:

$$P^{1,1}_{d23} = \frac{\partial S}{\partial Y_{X/S}} = 4.21, \quad P^{1,1}_{d25} = \frac{\partial S}{\partial S_f} = 1.53$$

Thus, the effect of the disturbance in $Y_{X/S}$ or S_f on the uncontrolled S is much less than in the first case where the growth is substrate limited. Therefore, when the substrate does not limit the cell growth during the fermentation process, the conventional turbidostat is also effective; especially when the disturbances in $Y_{X/S}$ and S_f are less important.

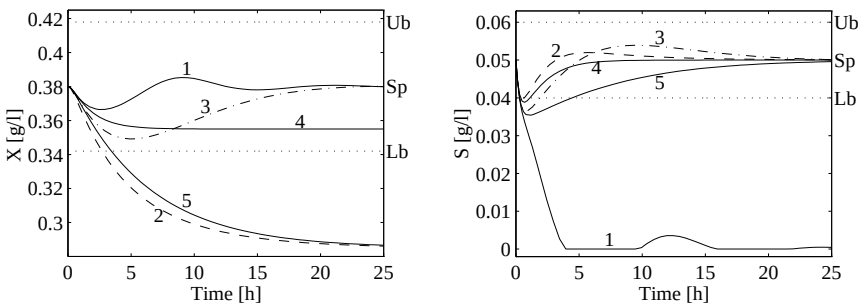
3.5 Simulation Results

In this section we present nonlinear simulation results to confirm the validity of the conclusions from the linear controllability analysis presented in the previous section. All simulations are for operating point No. I and a simple PI controller is used for the controlled output. Two alternative control actions are the dilution rate D and the feed substrate concentration S_f, the corresponding control laws are

$$D = \overline{D} + K_c\,[e(t) + 1/\tau_I \int_0^t e(t)\,dt] \tag{3.22}$$

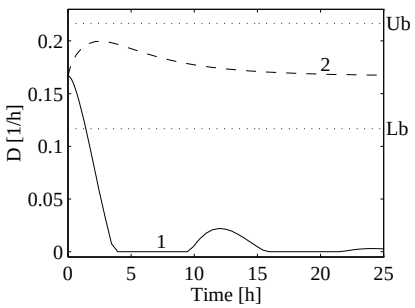
$$S_f = \overline{S_f} + K_c\,[e(t) + 1/\tau_I \int_0^t e(t)\,dt] \tag{3.23}$$

where $e(t) = \overline{X} - X$ (or $\overline{S} - S$). Because of the low order of the model (first-order or second-order transfer functions without time delays) considered in our case, ordinary controller tuning techniques, e.g., Ziegler-Nichols method, are not applicable. We therefore set the proportional gain (K_c) to give a manipulated input (control action) of reasonable magnitude, and set the integral time (τ_I) to get the acceptable response of the controlled output. The resulting controller tunings are given in Table 3.4.

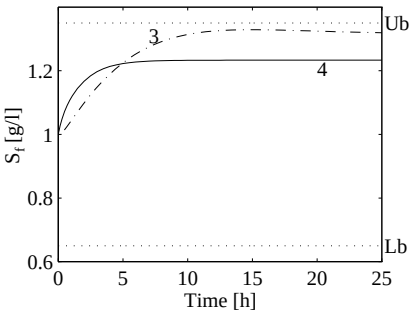


(a) Output X

(b) Output S



(c) Control action D



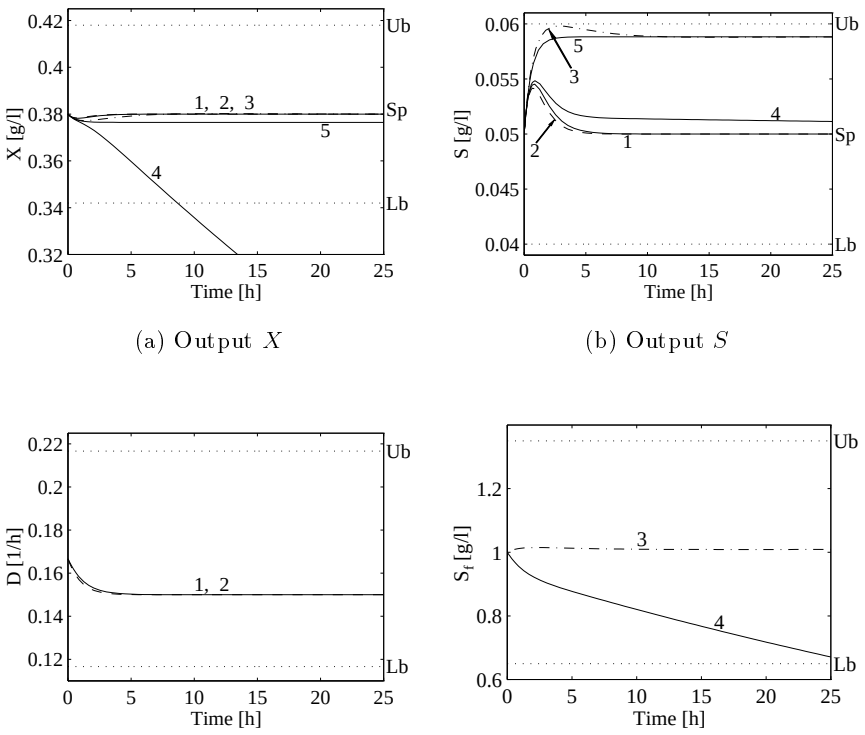
(d) Control action S_f

Figure 3.8: Time responses to a 25% step disturbance in $Y_{X/S}$

Table 3.4: Controller tunings and symbols used in Figure 3.8 and Figure 3.9

line type	configuration	K_c	$\tau_I[h]$
curves 1	Conventional turbidostat ($D \rightarrow X$ – configuration)	-2	0.5
curves 2	Conventional nutristat ($D \rightarrow S$ – configuration)	1	0.5
curves 3	Concentration turbidostat ($S_f \rightarrow X$ – configuration)	4	4
curves 4	Concentration nutristat ($S_f \rightarrow S$ – configuration)	4	0.5
curves 5	No control action	0	

Ub — Upper bound, Lb — Lower bound, Sp — Set point



(a) Output X

(b) Output S

(c) Control action D

(d) Control action S_f

Figure 3.9: Time responses to a 10% step disturbance in μ_m

Figure 3.8 shows step responses to a disturbance of 25% decrease in the yield factor ($Y_{X/S}$) from 0.4 to 0.3 [g/g]. Figure 3.9 shows step responses to a disturbance of 10% decrease in specific growth rate (μ_m) from 0.5 to 0.45.

From the simulations we find:

No control (curves 5): The system cannot be left uncontrolled because of disturbances in $Y_{X/S}$ that results in cell concentration exceeding its bound (see Figure 3.8(a)).

Conventional turbidostat (curves 1): For a disturbance in $Y_{X/S}$ the conventional turbidostat is unacceptable because the uncontrolled output S exceeds its bound. In fact S goes to zero and so does the dilution rate D (see Figure 3.8(b)–(c)), which means there is no production.

Conventional nutristat (curves 2): For a disturbance in $Y_{X/S}$ the conventional nutristat is poor because the uncontrolled output X exceeds its bound (see Figure 3.8(a)).

Concentration turbidostat (curves 3): Overall, the *best* performance is obtained by using the concentration turbidostat. This confirms the linear controllability analysis results. We see that with this configuration both the cell concentration and substrate concentration remain within their allowable bounds (as shown in Figure 3.8(a)–(b) and Figure 3.9(a)–(b)), and so does the feed substrate concentration (S_f) without violating the input constraints (see Figure 3.8(d) and Figure 3.9(d)).

Concentration nutristat (curves 4): For a disturbance in μ_m the concentration nutristat (curves 4 in Figure 3.9) is unacceptable because the uncontrolled output X exceeds its bound and eventually the reaction will stop: The input concentration S_f is continuously lowered in order to try to keep S constant. However, because the gain is zero at steady state, it is not possible to keep S constant in the long run, and eventually S_f will reach 0 and the reaction stops (in fact, we may get washout before this occurs since washout occurs when S_f falls below 0.05 g/l; recall the plot in Figure 3.5(b)). From the fermentation technology point of view, when a continuous bioreactor is operated at a steady state, the variation in the substrate concentration caused by the feed substrate concentration is exactly balanced or consumed by the growth of cell mass, and results in no net change in the substrate concentration in the reactor finally, i.e. S is independent of S_f. On the other hand, S is dependent of the maximum specific growth rate μ_m. Thus the effect of the disturbance in μ_m on S can not be compensated by changing S_f. Similar results with respect to disturbances in K_s and D are obtained (not

shown in the paper). Therefore this control configuration is not feasible in most cases.

A careful comparison shows that *all* these results are consistent with the linear controllability analysis results in the previous section.

3.6 Conclusions

At the substrate-limited growth conditions (operating point No. I), the controllability analysis results indicate that using S_f to control X (the concentration-turbidostat) is the best control configuration. This conclusion is consistent with the result of Menawat and Balachander (1991). The conventional turbidostat can not be used at this operating point because the cell concentration (X) is insensitive to changes in the dilution rate (D). The conventional nutristat is also not recommended from the controllability analysis, and it has the additional problem that it is difficult to accurately measure low values of substrate concentration (Agrawal and Lim, 1984).

At higher dilution rates where the cell growth is not substrate limited (operating point No. II), the conventional nutristat is slightly better than the other configurations, consistent with the results of Agrawal and Lim (1984). However there is no big difference among the first three control configurations. Thus the conventional turbidostat is also effective.

The concentration nutristat where S_f is used to control S (proposed by Menawat and Balachander, 1991) is unacceptable at all operating points because S_f has no steady-state effect on S.

In this paper we didn't consider the measurement problem which is one of the main obstacles for effective control of bioreactors. However, a number of secondary measurements are often available, such as temperature, pH, CO_2 and O_2 in the exhaust gas, which may be used to estimate the concentrations of cell mass and substrate.

In this paper, we have generalized the partial disturbance gain, which was first proposed by Skogestad and Wolff (1992) confined to the case that only one output is uncontrolled with other loops closed, to the common case with more outputs uncontrolled.

The simulation results are consistent with the linear controllability analysis results. This shows that the partial disturbance gain is an effective tool for controllability analysis. The main advantage with a controllability analysis is that

it is independent of controller parameter tuning and makes detailed simulations unnecessary.

Nomenclature

C	controller transfer function matrix	
D	dilution rate	[h^{-1}]
D_S	dilution rate of the concentrated substrate stream	[h^{-1}]
D_w	dilution rate of the sterile water stream	[h^{-1}]
d	disturbance	
F	flow rate	[l/h]
F_S	flow rate of the concentrated substrate stream	[l/h]
F_w	flow rate of the sterile water stream	[l/h]
G	plant transfer function matrix	
G_d	disturbance transfer function matrix	
K_c	controller gain	[h]
K_s	saturation constant in Monod model	[g/l]
P	cell productivity	[g cells/(h $\cdot$ l)]
P_d	partial disturbance gain	
$\|P_d\|_{i2}$	induced 2-norm of P_d	
r	cell growth rate	[g cells/(h $\cdot$ l)]
s	Laplace variable	
S	substrate concentration	[g/l]
S_{ef}	effective feed substrate concentration	[g/l]
S_f	feed substrate concentration	[g/l]
S_{sp}	setpoint value of S	[g/l]
u	plant input	
V	volume	[l]
X	cell concentration	[g/l]
X_{sp}	setpoint value of X	[g/l]
$Y_{X/S}$	yield coefficient	[g cells/ g substrate]
y	plant output	

Greek Symbols

$\bar{\sigma}(P_d)$	largest singular value of P_d	
τ_I	integral time	[h]
ω	frequency	[rad/h]
$\mu(S)$	specific growth rate	[h^{-1}]
μ_m	maximum specific growth rate	[h^{-1}]

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Appendix. Derivation of the partial disturbance gain

Consider a linear model of the form

$$y(s) = G(s)u(s) + G_d(s)d(s) \tag{3.24}$$

We here assume that the outputs y_2 are perfectly controlled by using u_2 and use the following definition of the partial disturbance gain for the uncontrolled outputs y_1:

$$P_{d1} \stackrel{\text{def}}{=} \left(\frac{\partial y_1}{\partial d}\right)_{\{y_2=0\} \text{ using } \{u_2\}} \tag{3.25}$$

As shown in Figure 3.2, we rearrange and partition the model $y = Gu + G_d d$ such that the first part of y contains the uncontrolled outputs (y_1), and the first part of u contains the unused inputs (u_1), whereas the remaining outputs y_2 are controlled using the remaining inputs u_2. We then have

$$y_1 = G_{11}u_1 + G_{12}u_2 + G_{d1}d \tag{3.26}$$

$$y_2 = G_{21}u_1 + G_{22}u_2 + G_{d2}d \tag{3.27}$$

In order to evaluate the effect of d on y_1 when y_2 is perfectly controlled using u_2, we set $y_2 = 0$ and solve for u_2 in Eq. (3.27) to get

$$u_2 = -G_{22}^{-1}G_{d2}d - G_{22}^{-1}G_{21}u_1 \tag{3.28}$$

We have here assumed that G_{22} is square and invertible, otherwise we may replace G_{22}^{-1} by the pseudo-inverse, $G_{22}^{\dagger}$. By substituting Eq. (3.28) into Eq. (3.26) we get

$$y_1 = (G_{d1} - G_{12}G_{22}^{-1}G_{d2})d + (G_{11} - G_{12}G_{22}^{-1}G_{21})u_1 \tag{3.29}$$

Then we have derived the expression of partial disturbance gain for y_1 with y_2 perfectly controlled using u_2,

$$\boxed{P_{d1} = G_{d1} - G_{12}G_{22}^{-1}G_{d2}} \tag{3.30}$$

This expression of the partial disturbance gain is general (no assumptions on G_{11}) and similar to the expression presented by Havre and Skogestad (1996).

If G_{11} is also square and invertible, let

$$\hat{G}^{-1} = \begin{bmatrix} G_{11} & \\ & G_{22} \end{bmatrix}^{-1} = \begin{bmatrix} G_{11}^{-1} & \\ & G_{22}^{-1} \end{bmatrix} \tag{3.31}$$

Then we have

$$G\hat{G}^{-1} = \begin{bmatrix} I & G_{12}G_{22}^{-1} \\ G_{21}G_{11}^{-1} & I \end{bmatrix} \tag{3.32}$$

Therefore an alternative expression of the partial disturbance gain is derived,

$$\boxed{P_{d1} = G_{d1} - [G\hat{G}^{-1}]_{12}G_{d2}} \tag{3.33}$$

which was first proposed by Zhao and Skogestad (1995).

We introduce a matrix $\Delta^{-1} = (G_{11} - G_{12}G_{22}^{-1}G_{21})^{-1}$ which is called the *Schur complement* of G_{22} in G. Assuming G_{22}^{-1} and Δ^{-1} exist we have from the Matrix Inversion Lemma (e.g. Zhou et al., 1996, p. 23),

$$G^{-1} = \begin{bmatrix} G_{11} & G_{12} \\ G_{21} & G_{22} \end{bmatrix}^{-1} = \begin{bmatrix} \Delta^{-1} & -\Delta^{-1}G_{12}G_{22}^{-1} \\ -G_{22}^{-1}G_{21}\Delta^{-1} & G_{22}^{-1} + G_{22}^{-1}G_{21}\Delta^{-1}G_{12}G_{22}^{-1} \end{bmatrix} \tag{3.34}$$

With $G_d = [G_{d1} \quad G_{d2}]^T$ we may rewrite Eq. (3.30) as follows,

$$\begin{aligned} P_{d1} &= \Delta\Delta^{-1}(G_{d1} - G_{12}G_{22}^{-1}G_{d2}) = \Delta(\Delta^{-1}G_{d1} - \Delta^{-1}G_{12}G_{22}^{-1}G_{d2}) \\ &= ([G^{-1}]_{11})^{-1}[G^{-1}G_d]_1 \end{aligned} \tag{3.35}$$

Special case, *one* uncontrolled output y_1: In this case, G_{11} is a scalar and then Δ^{-1} is also a scalar. Eq. (3.35) becomes

$$\boxed{P_{d1} = \frac{[G^{-1}G_d]_1}{[G^{-1}]_{11}}} \tag{3.36}$$

which was first proposed by Skogestad and Wolff (1992).

Chapter 4

Preliminary Study on Propionibacteria Fermentation Kinetics

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4.1 Introduction

From a control engineering point of view, an essential prerequisite for good supervision and operation of bioreactors in a rational way is an understanding of the process (fermentation) behavior, i.e. the knowledge of the process dynamics is of major importance. As fermentation processes involve living organisms in which a large number of biochemical reactions take place and microorganisms themselves have complex regulatory systems, a study of the properties and behavior of the living microorganisms is needed.

This thesis is mainly written for readers with a background in control engineering. Therefore this chapter contains a very brief introduction to the basic knowledge of cell growth and function for understanding biotechnical process dynamic behavior. Furthermore, the general characteristics of Propionibacteria (genus *Propionibacterium*), which is the particular organism used in this work presented throughout the rest chapters of this thesis, are given in Section 4.4 and some simple experimental results for the fermentation kinetics of propionibacteria are presented in Section 4.5.

4.2 A Little Microbiology

A living microorganism may be viewed in an approximate conceptual sense as an expanding chemical reactor which takes in chemical species called nutrients from its environment, grows, reproduces, and releases products into its surroundings. A key feature of a living microorganism is its ability to organize molecules and chemical reactions into specific structures and systematic sequences, and finally to replicate itself. The term *metabolism* is used to refer to all the chemical processes taking place mainly within a cell.

The chemicals from the environment which a cell utilizes are called *nutrients* or *substrates*. Nutrients are taken up into the cell and are converted into cell constituents and energy. This process by which a cell is built up from the simple nutrients obtained from its environment is called *anabolism*. Because anabolism results in the biochemical synthesis of new cell material, it is often called *biosynthesis*. Biosynthesis is an energy-requiring process, and each cell must thus have a means of obtaining energy.

Chemicals used as energy source are broken down into simpler constituents, and as this breakdown occurs, energy is released. The process by which chemicals

are broken down and energy released is called *catabolism*. In addition to biosynthesis, cells need energy for other cell functions, such as cell movement (motility) and transport of nutrients as well as maintaining concentration gradients across cell membrane. Note that in anabolism, nutrients from the environment are converted into cell components, whereas in catabolism, nutrients from the environment are converted into *metabolic products* or *products* which refer to the substances, typically organic compounds, different from cell material.

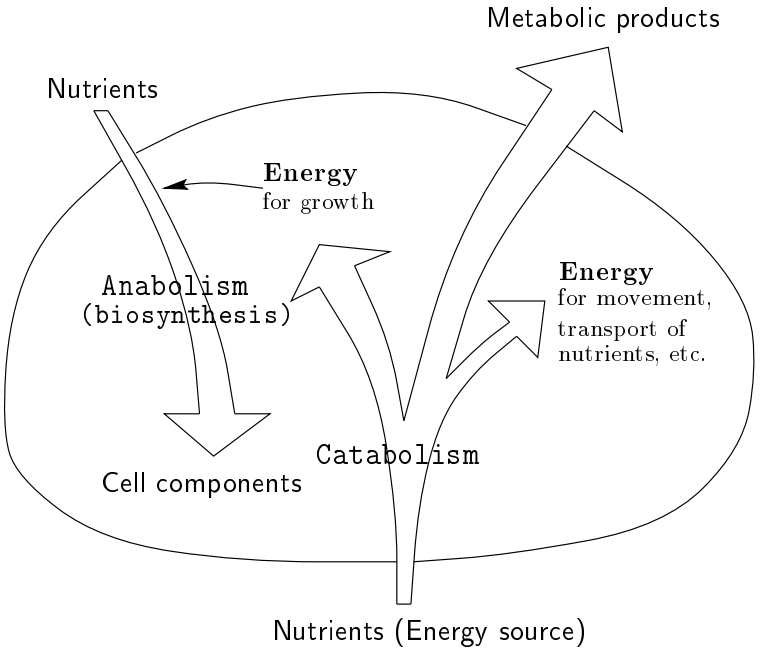


Figure 4.1: A simplified overview of cell metabolism *(Based on Brock and Madigan (1991))*

As shown in Figure 4.1, there are two basic kinds of chemical transformation processes occurring in cells, the breaking-down processes in which catabolic reactions supply energy needed for cell functions by means of breakdown of organic compounds, and the building-up processes in which anabolic reactions bring about the synthesis of cell components from simpler molecules. Metabolism is thus the collective result of anabolic and catabolic reactions. In other words, during metabolism, cells take in nutrients, convert them into cell components, and excrete metabolic products into environment. The relative rates of nutrient utilization, cell growth, and release of products depend strongly on the type of

cells involved, and on the type and accessibility of nutrients.

4.3 The Mathematics of Growth (Monod Growth kinetics)

Although many analyses of microbial growth can be done graphically, for further purposes it is convenient to use differential equations to describe the cell growth and nutrient utilization:

$$\frac{dX}{dt} = \mu X \tag{4.1}$$

$$\frac{dS}{dt} = -\frac{\mu X}{Y_{X/S}} \tag{4.2}$$

Here X is the cell concentration, S substrate concentration, and μ the specific cell growth rate. $Y_{X/S}$ is the yield coefficient for the particular microorganism on the particular substrate, and is defined as:

$$Y_{X/S} = \frac{\text{mass of cells formed}}{\text{mass of substrate consumed}} = \frac{\Delta X}{\Delta S} \tag{4.3}$$

So $Y_{X/S}$ is a measure of the efficiency with which cells convert nutrient to more cell material. Considering different substrates S_i in the medium, different corresponding cell growth yield coefficients Y_{X/S_i} may be defined. In this thesis, $Y_{X/L}$ denotes the yield coefficient on the substrate lactic acid, and $Y_{X/Y}$ the yield coefficient on the substrate yeast extract-peptone.

In these equations the only modelling assumption is that the cell growth term (μX) and the substrate consumption term ($\frac{\mu X}{Y_{X/S}}$) are proportional to the cell concentration X. This assumption has been validated many times and has become commonly accepted since Monod introduced it in 1942 (Bastin and Dochain, 1990).

A functional relationship between the specific cell growth rate μ and nutrient concentration was proposed by Monod in 1942. This function represents a saturation process described by the Monod model:

$$\mu = \mu_m \frac{S}{K_s + S} \tag{4.4}$$

Here S is the concentration of the limiting nutrient[†]. μ_m is the maximum specific cell growth rate in a given medium at specified temperature and pH, and μ_m

[†]It is usual to refer to a component of the medium as a *limiting nutrient*, if an increase in its concentration causes an increase in cell growth rate.

is achievable when $S \gg K_s$ and the concentrations of all other nutrients are present in excess and unchanged. K_s is a substrate-specific saturation constant, and numerically equal to the limiting substrate concentration at which half the maximum specific growth rate is obtained ($\mu = 0.5\mu_m$). In biological terms K_s is inversely proportional to the affinity of microorganism for its substrate. K_s is equivalent to the Michaelis constant in enzyme kinetics, and the K_s-value for an enzyme depends on the particular substrate and also on environmental conditions such as pH, temperature, and ionic strength.

The Monod model is based on empirical observations but it is frequently rationalized by analogy with Michaelis-Menten enzyme kinetics with the hypothesis that a single rate-limiting enzyme-catalyzed step controls growth rate. Many other models have been proposed to discuss the dependence of cell growth rate on nutrient concentration (summarized by Bastin and Dochain, 1990, pp. 339–342), but the Monod model is most commonly used (Wang et al., 1979).

4.4 General Characteristics of Propionibacteria

The Propionibacteria (genus *Propionibacterium*) were first discovered as inhabitants of Swiss (Emmenthaler) cheese, and extensive studies on their growth dynamics, nutritional requirements, metabolism and miscellaneous metabolic activities have been carried out. A series of comprehensive reviews were given by Hettinga and Reinbold (1972a; 1972b; 1972c). However, only the most important characteristics of propionibacteria are given below.

Propionibacteria may be characterized, in general, as being Gram-positive, pleomorphic, nonsporulating rods, nonmotile and facultative anaerobes (Sneath et al., 1986). Their nutritional requirements are complex, and they usually grow rather slowly. The optimum pH range for growth is between 6 and 7, and at pH $<$ 4.5 there is practically no growth. Growth and fermentation of propionibacteria are inhibited by acidic pHs and the major fermentation product, propionic acid (Lewis and Yang, 1992). The growth of propionibacteria can be readily described by the simple Monod model (Hettinga and Reinbold, 1972a; Lee et al., 1974).

Propionibacteria ferment lactic acid with the production of propionic acid, acetic acid, and CO_2. The overall stoichiometry for the formation of propionic acid from lactic acid by the action of propionibacteria is

$$3\,\text{Lactic acid} \longrightarrow 2\,\text{Propionic acid} + 1\,\text{Acetic acid} + 1\,CO_2 \qquad (4.5)$$

It is the fermentation of lactic acid to propionic acid that is important in Swiss cheese manufacture. The fermentative production of CO_2 produces the characteristic holes and the presence of propionic acid is at least partly responsible for the unique flavor of the cheese. Propionibacteria have long been used in the dairy industry. Although they are used primarily in cheese production, they are also utilized for commercial production of vitamin B_{12} and propionic acid.

4.5 Experimental Study on the Kinetics of Cell Growth

Although the nutritional requirements and metabolic properties of propionibacteria have been studied extensively, very little quantitative information is available about the fermentation kinetics of Propionibacteria growing on a complex medium yeast extract-peptone with lactic acid as the energy source, especially about the saturation constant (K_s) in the Monod model. To our knowledge, there is only one paper by Jain et al. (1991) to discuss the effect of yeast extract on propionic acid production in batch process. The objective of this section is to study the fermentation kinetics and estimate kinetic parameters when lactic acid and yeast extract-peptone are used as growth-limiting substrate, respectively. The laboratory results for batch cultivation of propionibacteria are presented. A discussion is given on the fermentation kinetics of cell growth and substrate uptake, especially on the validity of using the simple Monod model to describe the growth of propionibacteria. The experimental results also provide some insights into estimation of kinetic parameters, such as the specific growth rate, the Monod saturation constant and yield coefficients.

4.5.1 Materials and methods

Microorganism. An industrial *Propionibacterium shermanii* strain, provided by the Biotechnology group in SINTEF Applied Chemistry, was used in this study.

Medium. The medium used for the first experiment was prepared from tap water and contained 25 g/l yeast extract-peptone mixture (YEP) and various amounts of sodium lactate (0.75–8 g/l); the medium used for the second experiment was prepared from tap water and contained 4 g/l sodium lactate (LA) and various amounts of yeast extract-peptone (2.5–15 g/l). All media were autoclaved at $120^{\circ}C$ for 25 min before use. A detailed composition of the media is presented

in Table 4.1.

Table 4.1: Composition of media

Experiment	Lactic acid	Yeast extract-Peptone	Fermentation time
1	8 g/l in bottle 1a 4 g/l in bottle 1b 1.5 g/l in bottle 1c 0.75 g/l in bottle 1d	25 g/l in all bottles 1a–1d	48 h
2	4 g/l in all bottles 2a–2d	15 g/l in bottle 2a 10 g/l in bottle 2b 5 g/l in bottle 2c 2.5 g/l in bottle 2d	35 h

Analytical methods. At appropriate time intervals (depending on the fermentation rate), 10-ml liquid samples of fermented broth were taken. Cell concentration in fermentation broth was monitored by measuring the optical density at 660 nm (OD_{660}) of the suspension using a spectrophotometer. Uninoculated fresh medium was used as the reference solution for zero correction in all determinations. It was found that OD was proportional to dry cell weight when OD was less than 0.4. Sample was diluted with distilled water if the OD was greater than 0.4. Concentrations of lactic, propionic and acetic acids in sample solutions were analyzed with high-performance liquid chromatography (HPLC).

Batch cultivation of *P. shermanii* on different initial lactic acid concentrations

Considering lactic acid as the growth-limiting substrate, four fresh media were prepared with different initial lactic acid concentrations as described in Table 4.1. Two successive inoculations were made in the fermentation medium prior to the final batch cultivation. At first, a bottle containing 500 ml, 25 g/l yeast extract-peptone and 8 g/l lactate, medium was inoculated with 5 ml of *P. shermanii* cells and incubated for 72 h in an incubator at a controlled temperature of $30^{\circ}C$. 5 ml of this culture was then used to inoculate another bottle containing same amount and composition of fresh medium as the first bottle, and incubated at $30^{\circ}C$ for 43 h. This second culture was finally used for inoculation of batch fermentation

experiments: as numbered in Table 4.1, bottle 1a (500 ml fresh medium containing 8 g/l lactic acid) and bottle 1b (500 ml fresh medium containing 4 g/l lactic acid) were inoculated by 50 ml of this second culture, respectively; the remaining 2 bottles 1c (containing 1.5 g/l lactic acid) and 1d (containing 0.75 g/l lactic acid) were inoculated by 25 ml of this second culture, respectively. The batch cultivation of *P. shermanii* was then conducted in 4 bottles, with different initial lactic acid concentration, but the same yeast extract-peptone concentration (25 g/l) which was in excess, in an incubator at a controlled temperature of $30^{\circ}C$ for 48 h without agitation. During the fermentation process, no external pH control was used because the acid products formed were about equimolar with the lactic acid consumed (recall Eq. (4.5)). In our experiments, the pH change in the culture due to fermentation of lactate was small—pH values ranged between 6.2 and 6.7.

Batch cultivation of *P. shermanii* on different initial yeast extract-peptone concentrations

In this experiment, lactic acid was supplied in excess, 4 g/l. Four fresh media were prepared with different initial yeast extract-peptone concentrations, as described in Table 4.1, in order to investigate the effect of yeast extract-peptone in limited amount on the growth kinetics of *P. shermanii*. A bottle containing 500 ml fresh medium composed of 25 g/l yeast extract-peptone and 8 g/l lactate was first inoculated by 10 ml of *P. shermanii* cells and incubated for 41 h in an incubator at a controlled temperature of $30^{\circ}C$. 25 ml inoculum of this culture was then transfered to each of four bottles containing 500 ml fresh medium with different initial yeast extract-peptone concentration, 15 g/l, 10 g/l, 5 g/l, 2.5 g/l, and thus the second batch fermentation experiment was carried out in these four bottles in an incubator under the same fermentation conditions as described for the first experiment and lasted for 35 h.

4.5.2 Results and discussion

Based on mass balances, the increase in cell mass is given by

$$\frac{dX}{dt} = \text{cell growth} - \text{outflow} - \text{cell death} \tag{4.6}$$

and the change in substrate concentration is given by

$$\frac{dS}{dt} = \text{inflow} - \text{outflow} - \text{consumption} - \text{maintenance} \tag{4.7}$$

Because during the batch fermentation process there are no substrate feed and product withdraw, and assuming that the decay term accounting for the natural death of microorganisms and the maintenance term accounting for a part of the substrate used for cell survival can be omitted, the dynamic equations for cell growth and substrate utilization then have the similar form as Eqs. (4.1–4.2):

$$\frac{dX}{dt} = \mu X \tag{4.8}$$

$$\frac{dS_L}{dt} = -\frac{\mu X}{Y_{X/L}} \tag{4.9}$$

$$\frac{dS_Y}{dt} = -\frac{\mu X}{Y_{X/Y}} \tag{4.10}$$

The growth kinetics of *P. shermanii* may be represented by the Monod model:

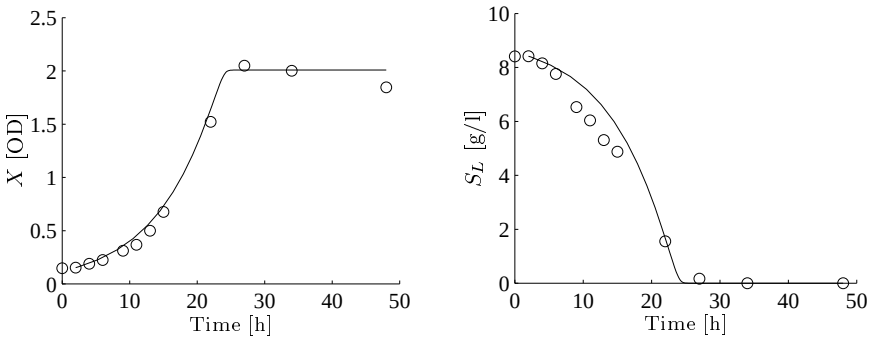
$$\mu = \mu_m \frac{S}{K_s + S} \tag{4.11}$$

where S may stand for S_L and K_s for K_{sL} when considering lactic acid as growth-limiting substrate, whereas S denotes S_Y and K_s denotes K_{sY} if yeast extract-peptone acts as limiting substrate. The maximal specific cell growth rate μ_m here is calculated by the least square method based on experimental data and the saturation constant K_s is estimated around the substrate concentration at which $\mu = 0.5\mu_m$. The yield coefficient is calculated according to its definition given by Eq. (4.3), that is,

$$Y_{X/S} = \frac{X_f - X_i}{S_i - S_f}$$

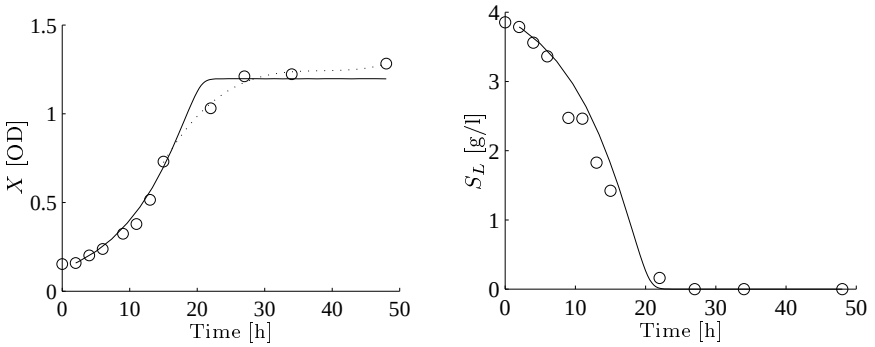
where the subscript i means initial time (just after inoculation) and f final time.

Figures 4.2–4.5 show the results of first batch cultivation of *P. shermanii* in four 500-ml bottles with different initial lactic acid concentration but the same initial yeast extract-peptone concentration. For cells growing on the high initial lactic acid concentration, the Monod kinetics describes adequately the course of cell growth as shown in Figure 4.2(a). For cells growing on lower initial lactic acid concentrations, during the first 15 hours when lactic acid is served as



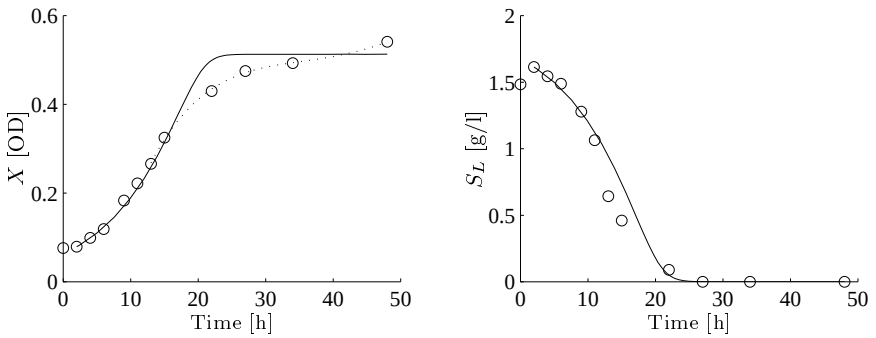
(a) Cell growth curve (b) Lactic acid utilization profile

Figure 4.2: Batch cultivation of *P. shermanii* in 8 g/l LA, 25 g/l YEP medium (experiment 1a). $\mu_m = 0.125$ h^{-1}, $K_{sL} = 0.27$ g/l and $Y_{X/L} = 0.22$. Symbols: o, measured data; —, predicted data by Eqs. (4.8), (4.11) for cell concentration and Eqs. (4.9), (4.11) for lactic acid concentration.



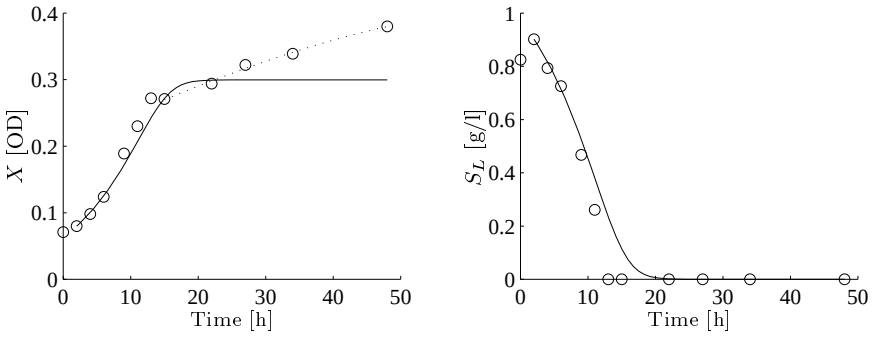
(a) Cell growth curve (b) Lactic acid utilization profile

Figure 4.3: Batch cultivation of *P. shermanii* in 4 g/l LA, 25 g/l YEP medium (experiment 1b). $\mu_m = 0.125$ h^{-1}, $K_{sL} = 0.27$ g/l and $Y_{X/L} = 0.27$. Symbols: o, measured data; ..., polynomial fitting curve by Eq. (4.15); —, predicted data by Eqs. (4.8), (4.11) for cell concentration and Eqs. (4.9), (4.11) for lactic acid concentration.



(a) Cell growth curve (b) Lactic acid utilization profile

Figure 4.4: Batch cultivation of *P. shermanii* in 1.5 g/l LA, 25 g/l YEP medium (experiment 1c). $\mu_m = 0.13$ h^{-1}, $K_{sL} = 0.27$ g/l and $Y_{X/L} = 0.27$. Symbols: o, measured data; ..., polynomial fitting curve by Eq. (4.16); —, predicted data by Eqs. (4.8), (4.11) for cell concentration and Eqs. (4.9), (4.11) for lactic acid concentration.



(a) Cell growth curve (b) Substrate utilization profile

Figure 4.5: Batch cultivation of *P. shermanii* in 0.75 g/l LA, 25 g/l YEP medium (experiment 1d). $\mu_m = 0.15$ h^{-1}, $K_{sL} = 0.27$ g/l and $Y_{X/L} = 0.24$. Symbols: o, measured data; ..., polynomial fitting curve by Eq. (4.17); —, predicted data by Eqs. (4.8), (4.11) for cell concentration and Eqs. (4.9), (4.11) for lactic acid concentration.

carbon source and energy source, the predicted data based on the Monod kinetics by Eqs. (4.8), (4.9) and (4.11) are in good agreement with the off-line measured cell OD and lactic acid concentration data. However, after lactic acid is exhausted, second growth phases are observed in these experiments where cells growing on lower initial lactic acid concentrations, as shown by the dotted curves in Figure 4.3(a)–4.5(a). Since the yeast extract-peptone is supplied in excess ($S_{iY} = 25$ g/l) and cell growth proceeds in complex medium, cells are able to utilize some components in yeast extract-peptone as carbon and energy source after lactic acid is depleted and continually grow. In a precise manner, the dynamic equations may be rewritten as:

$$\frac{dX}{dt} = \left(\frac{\mu_{mL} S_L}{K_{sL} + S_L} + \frac{\mu_{mY} S_Y}{(K_{sY} + S_Y)(K_{IL} + S_L)} \right) X \tag{4.12}$$

$$\frac{dS_L}{dt} = -\frac{1}{Y_{X/L}} \frac{\mu_{mL} S_L}{K_{sL} + S_L} X \tag{4.13}$$

$$\frac{dS_Y}{dt} = -\frac{1}{Y_{X/Y}} \frac{\mu_{mY} S_Y}{(K_{sY} + S_Y)(K_{IL} + S_L)} X \tag{4.14}$$

where K_{IL} accounts for the inhibition effect by the presence of lactic acid. However, as a matter of fact, yeast extract-peptone is a complex medium and then its concentration is unmeasurable in our simple batch fermentation experiments. Then the parameter K_{sY} in Monod kinetics can not be determined.

Mathematically, we can use a polynomial function to fit the experimental data as shown by the dotted curves in Figure 4.3(a)–4.5(a). In particular, by simulation work, we find the following formulae to describe cell growth as time proceeds.

Polynomial curve fitting to the experiment 1b data ($S_{iL} = 4$ g/l):

$$X = 3.88\, e{-}05\, t^3 - 4.48\, e{-}03\, t^2 + 1.73\, e{-}01\, t - 9.94\, e{-}01, \quad t \in [15 \quad 48] \tag{4.15}$$

Polynomial curve fitting to the experiment 1c data ($S_{iL} = 1.5$ g/l):

$$X = -2.25\, e{-}07\, t^4 + 4.29\, e{-}05\, t^3 - 2.87\, e{-}03\, t^2 + 8.36\, e{-}02\, t - 4.22\, e{-}01, \quad t \in [13 \quad 48] \tag{4.16}$$

Polynomial curve fitting to the experiment 1d data ($S_{iL} = 0.75$ g/l):

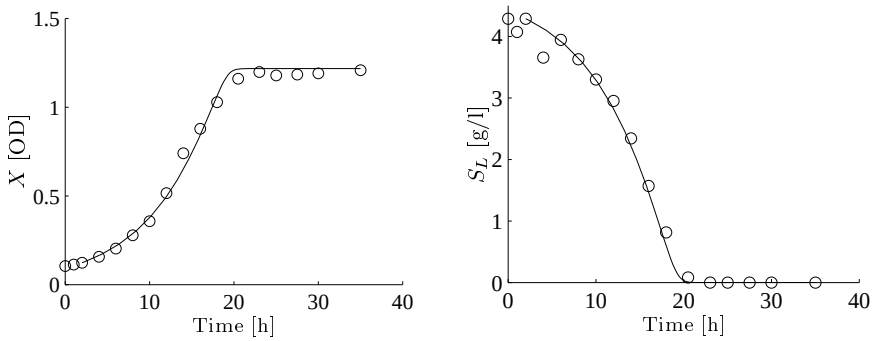
$$X = -2.60\, e{-}07\, t^3 - 3.88\, e{-}06\, t^2 + 4.41\, e{-}03\, t + 2.06\, e{-}01, \quad t \in [15 \quad 48] \tag{4.17}$$

The reason for no obvious second cell growth period observed in the experiment 1a ($S_{iL} = 8$ g/l) is that at high initial lactic acid concentration, much more cells are produced and then the yeast extract-peptone is consumed rapidly. Consequently, when 8 g/l lactic acid is used up, no enough yeast extract-peptone is available to be utilized as carbon and energy source for cell growing successively.

One thing should be indicated here, that is about estimating K_s value. Generally, it is difficult to estimate K_s value in batch culture fermentations since the transition from exponential to stationary phase is usually quite rapid or abrupt. It is possible however, to employ initial rate kinetics where low initial substrate levels are employed. Therefore, the K_{sL} value is estimated from the experiment 1c data where lower initial lactic acid concentration is used ($S_{iL} = 1.5$ g/l).

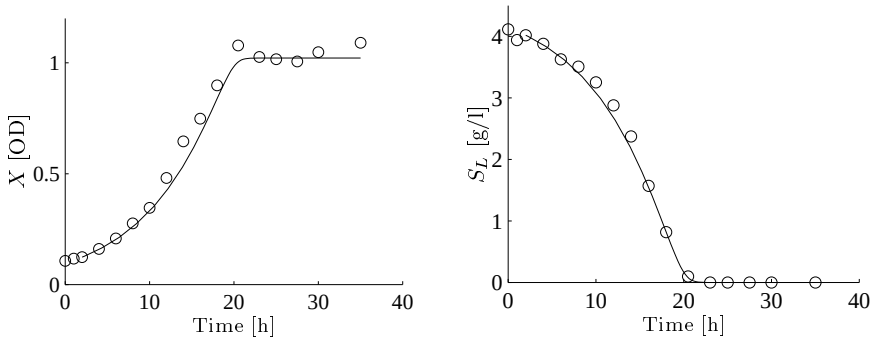
Figures 4.6–4.9 show the results of the batch cultivation of *P. shermanii* on different initial yeast extract-peptone concentration but the same lactic acid concentration. By comparing the experiment 2a results (where $S_{iL} = 4$ g/l and $S_{iY} = 15$ g/l) with the experiment 1b results (where $S_{iL} = 4$ g/l and $S_{iY} = 25$ g/l), we notice that the finally produced cells are in same level in spite of the yeast extract-peptone concentration decreased by a factor of 3/5. Similar behaviors are observed in experiments 2b and 2c. On the other hand, in experiments 2a–2c, cells stop growing after about 20 hours at which point the lactic acid is depleted (see Figures 4.6–4.8). Therefore, in regarding to experiments 2a–2c, cell growth actually is limited by lactic acid rather than by yeast extract-peptone. In other words, with respect to 4 g/l lactic acid supplied in the medium, $S_{iY} > 5$ g/l means that the yeast extract-peptone is supplied in excess and then can't act as limiting substrate to cell growth. Figures 4.6–4.8 depict very good fits of the measured data of cell OD and lactic acid concentration with the calculated data based on the Monod kinetics where lactic acid is assumed to be the growth-limiting substrate.

However, rather different phenomenon is presented in the experiment 2d where a low initial yeast extract-peptone concentration is used ($S_{iY} = 2.5$ g/l) (see Figure 4.9). It seems that in this case the cell growth is limited by the yeast extract-peptone instead of lactic acid, since cells stop growing after 20 hours although at that time lactic acid is still available. The predicted data, based on the Monod kinetics where yeast extract-peptone is assumed to be the growth-limiting substrate, are in good agreement with the measured data of cell OD. Since the yeast extract-peptone is complex medium, its concentration can not be measured during the fermentation process. Therefore, in Figure 4.9(b), the



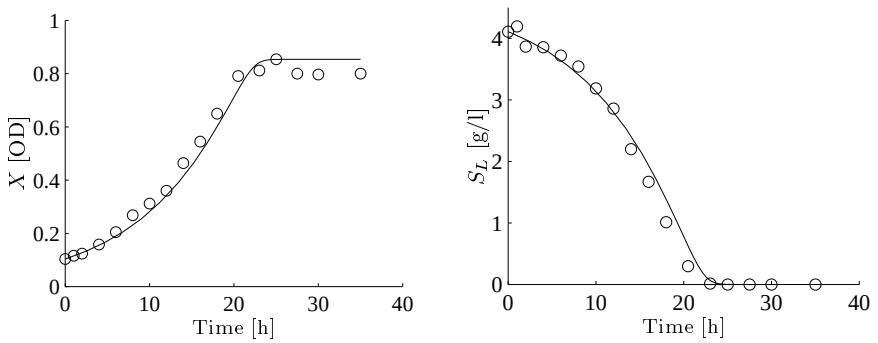
(a) Cell growth curve (b) Lactic acid utilization profile

Figure 4.6: Batch cultivation of *P. shermanii* in 15 g/l YEP, 4 g/l LA medium (experiment 2a). $\mu_m = 0.149$ h^{-1}, $K_{sL} = 0.27$ g/l and $Y_{X/L} = 0.255$. Symbols: o, measured data; —, predicted data by Eqs. (4.8), (4.11) for cell concentration and Eqs. (4.9), (4.11) for lactic acid concentration.



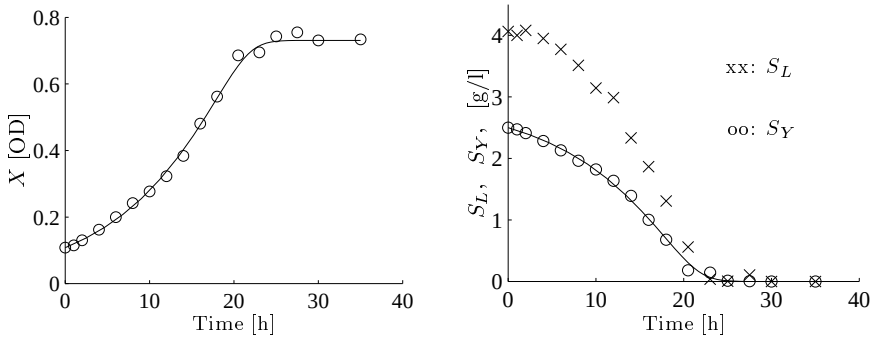
(a) Cell growth curve (b) Lactic acid utilization profile

Figure 4.7: Batch cultivation of *P. shermanii* in 10 g/l YEP, 4 g/l LA medium (experiment 2b). $\mu_m = 0.132$ h^{-1}, $K_{sL} = 0.27$ g/l and $Y_{X/L} = 0.223$. Symbols: o, measured data; —, predicted data by Eqs. (4.8), (4.11) for cell concentration and Eqs. (4.9), (4.11) for lactic acid concentration.



(a) Cell growth curve (b) Lactic acid utilization profile

Figure 4.8: Batch cultivation of *P. shermanii* in 5 g/l YEP, 4 g/l LA medium (experiment 2c). $\mu_m = 0.107$ h^{-1}, $K_{sL} = 0.27$ g/l and $Y_{X/L} = 0.183$. Symbols: o, measured data; —, predicted data by Eqs. (4.8), (4.11) for cell concentration and Eqs. (4.9), (4.11) for lactic acid concentration.



(a) Cell growth curve (b) Substrate utilization profile

Figure 4.9: Batch cultivation of *P. shermanii* in 2.5 g/l YEP, 4 g/l LA medium (experiment 2d). $\mu_m = 0.11$ h^{-1}, $K_{sY} = 0.34$ g/l and $Y_{X/Y} = 0.249$. Symbols: o, measured data for cell OD; x, measured data for lactic acid concentration; —, predicted data by Eqs. (4.8), (4.11) for cell concentration and Eqs. (4.10), (4.11) for yeast extract-peptone concentration.

yeast extract-peptone concentration data shown by the symbol 'o' are calculated from the measured data of cell concentration (OD), based on the assumption that the concentration of yeast extract-peptone, when used as limiting substrate, is proportional to the cell concentration, i.e. $S_Y = S_{iY} - \frac{X - X_i}{Y_{X/Y}}$

4.6 Concluding Remarks

The agreement between the batch experiment results and the calculated data based on the Monod kinetics is satisfactory, i.e. the Monod kinetics can describe adequately the growth course of *P. shermanii*. When considering lactic acid as growth-limiting substrate, the estimated value of the maximum specific growth rate μ_m ranges between 0.125 to 0.15 h^{-1}. This compares well with the results of Lee et al. (1974), who found $\mu_m = 0.141$ h^{-1} for *P. shermanii* on lactate. A saturation constant K_{sL} of 0.27 g/l is obtained, which is used in Monod kinetics for predicting cell growth course in experiments 1a–1d and 2a–2c, and the predicted data are in good agreement with the experimental data. When yeast extract-peptone limits cell growth, $\mu_m = 0.11$ h^{-1} and $K_{sY} = 0.34$ g/l are obtained from the experiment 2d data.

For cells growing on a low initial lactic acid concentration but yeast extract-peptone supplied in excess, we find that there is a second growth phase after lactic acid is exhausted because cells are able to utilize some components in yeast extract-peptone as carbon and energy source and continually grow.

From experiment results, we also notice that, corresponding to 4 g/l lactic acid supplied in the medium, when yeast extract-peptone is supplied in 2.5 g/l level or less, cell growth is possibly limited by yeast extract-peptone rather than by lactic acid. yeast extract-peptone is an excellent substrate for *P. shermanii*, but it is relatively expensive for industrial application. In order to decrease the cost of raw materials, yeast extract-peptone is preferred to be used as growth-limiting substrate. Therefore yeast extract-peptone concentration should be selected properly so that cell growth is really limited by yeast extract-peptone.

Nomenclature

LA	lactic acid
YEP	mixture of yeast extract and peptone

K_s	saturation constant in Monod model	[g/l]
K_{sL}	saturation constant when LA acts as limiting substrate	[g/l]
K_{sY}	saturation constant when YEP acts as limiting substrate	[g/l]
S	substrate concentration	[g/l]
S_L	substrate LA concentration	[g/l]
S_{iL}	initial substrate LA concentration	[g/l]
S_{fL}	final substrate LA concentration	[g/l]
S_Y	substrate YEP concentration	[g/l]
S_{iY}	initial substrate YEP concentration	[g/l]
S_{fY}	final substrate YEP concentration	[g/l]
X	cell concentration	[g/l]
X_i	initial cell concentration	[g/l]
X_f	final cell concentration	[g/l]
$Y_{X/S}$	cell growth yield coefficient	[g cells/ g substrate]
$Y_{X/L}$	cell growth yield coefficient on substrate LA	[g cells/ g LA]
$Y_{X/Y}$	cell growth yield coefficient on substrate YEP	[g cells/ g YEP]

Greek Symbols

μ	specific growth rate	[h^{-1}]
μ_m	maximum specific growth rate	[h^{-1}]
μ_{mL}	maximum specific growth rate when LA acts as limiting substrate	[h^{-1}]
μ_{mY}	maximum specific growth rate when YEP acts as limiting substrate	[h^{-1}]

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Chapter 5

High-concentration Cultivation of Propionibacteria in Continuous Bioreactor with Cross-flow Filtration. Part I: Modeling

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5.1 Introduction

As it is well known, dairy propionibacteria are commercially important in the production of "eyes" and typical flavors in Swiss-type cheeses. Few dairies prepare their own propionic cultures, and starter producers have developed the concentrated cultures in frozen or freeze-dried forms. Therefore there is a strong interest in the food industry to continuously obtain the propionibacteria with high concentration at the maximum production rate.

The work presented in the rest chapters of this thesis is based on an industrial propionibacteria fermentation process in a continuous bioreactor with cross-flow filtration. The general features of this industrial application are extensively studied around three topics: Modeling (discussed in this Chapter), Estimation (discussed in Chapter 6), and Control (in Chapter 7).

In this chapter, the first sections describe in details the characteristics of this industrial process and the derivation of the dynamic model of this process. In Section 5.4, simulation study results show by a sensitivity analysis the effect of the Monod parameter K_s-value on the steady state behavior of process and stress the importance of the knowledge about K_s for rational operation of this bioreactor, which is the main consideration in this chapter.

5.2 Description of Industrial Fermentation Process

A schematic diagram of an industrial continuous propionibacteria fermentation process is shown in Figure 5.1.

The operation objective of this continuous bioreactor with cross-flow filtration is to get constant and high concentration cultivation of propionibacteria. There are three feed streams into this continuous bioreactor as shown in Figure 5.1: yeast extract-peptone (a mixture of yeast extract and peptone) at the flow rate $D_{\text{in}1}$, lactic acid at the flow rate $D_{\text{in}2}$ and base solution at the flow rate $D_{\text{in}3}$.

The carbon source plays an important role in the growth of propionibacteria. In our case, lactic acid (LA) is used both as a carbon source for being incorporated directly into cellular material synthesis and as an energy source for cell growth and for other cell functions. The interesting thing is that lactic acid itself is an end product of fermentation for many bacteria. Propionibacteria are thus able to obtain energy anaerobically from a substance that other bacteria are producing by carrying out a secondary fermentation.

Yeast extract-peptone (YEP) is used as the nitrogen source for incorporation into cell mass synthesis. Yeast extract is an excellent substrate for many microorganisms as it contains amino acids and peptides, water-soluble vitamins, and carbonhydrates, and the composition of yeast extract varies, partly because the substrates used for yeast cultivation affect the quality of the yeast extract (Crueger and Crueger, 1990). Peptone can be utilized by many microorganisms but it is relatively expensive for industrial application. Peptone composition varies depending upon its origin. Propionibacteria have complex nutritional requirements and they require certain vitamins and minerals for growth and metabolism. Yeast extract-peptone is vital for the growth and metabolism of propionibacteria in that it provides nutrients and vitamins for propionibacteria so that these bacteria will best ferment lactic acid in the presence of yeast extract-peptone.

The base addition is used for compensating pH changes in the bioreactor. However, the pH change in the bioreactor due to fermentation of lactic acid is small because the acid products formed are about equimolar with the lactic acid consumed (recall Eq. (4.5)).

The major factor that limits the effective production of cell mass is an end-product inhibition caused by the acid. To overcome the inhibitory effect of propionic acid on bacterial growth, a continuous bioreactor combined with crossflow filtration (cell recycling) is quite efficient. As shown in Figure 5.1, the reactor

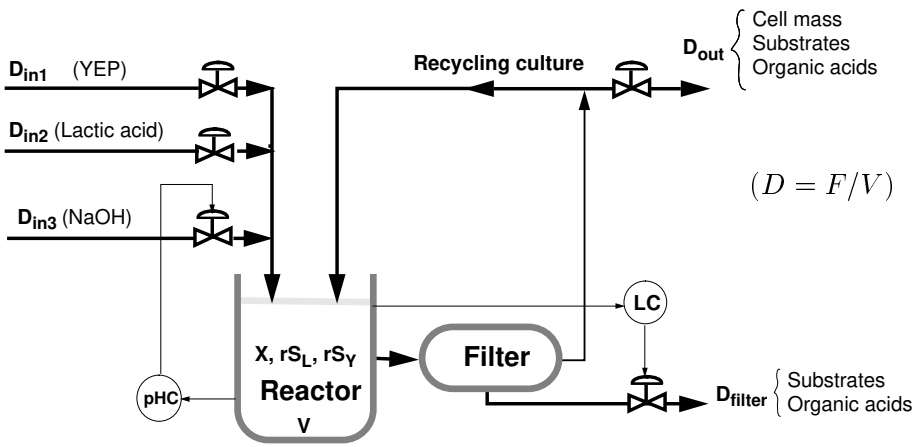


Figure 5.1: A continuous bioreactor with cross-flow filtration

effluent is fed to a filter which is used for increasing the cell concentration by bleeding off some used medium and produced organic acids at flow rate D_{filter}. After crossflow filtration, a part of the outlet stream from the filter, which includes the high concentration of cell mass, produced organic acids and used medium is recycled to the bioreactor. The rest part is pumped into the product tank at flow rate D_{out} for product purification. Here all flow rates F are normalized with respect to the bioreactor volume V, i.e. $D = F/V$.

5.3 Derivation of the Dynamic Process Model

The following assumptions are made for the further discussion:

1. The process is assumed to be in a completely mixed condition: this implies that the composition of the culture medium is homogeneous in the fermenter.

2. The concentrations of cell mass and substrates are uniform respectively in the whole loop (fermenter-filter) system.

3. The organic acids produced in this fermentation process, which are of no interest to us, are not considered.

4. The liquid density is constant.

5. The volume of the culture medium is constant.

Applying the principle of conservation of mass to the whole fermenter-filter system,

$$\text{Increase of storage} = \text{Inflow} - \text{Outflow} + \text{Rate of creation} \tag{5.1}$$

we then have the following equations,
the net accumulation of cell mass in the whole system [g/h]:

$$\frac{d(VX)}{dt} = -F_{\text{out}}X + \mu\, VX - k_d VX \tag{5.2}$$

the net accumulation of substrate yeast extract-peptone in the whole system [g/h]:

$$\frac{d(V S_Y)}{dt} = F_{\text{in1}} S_{fY} - (F_{\text{filter}} + F_{\text{out}})\, S_Y - \frac{\mu\, VX}{Y_{X/Y}} - k_{mY} VX \tag{5.3}$$

the net accumulation of substrate lactic acid in the whole system [g/h]:

$$\frac{d(VS_L)}{dt} = F_{\text{in2}} S_{fL} - (F_{\text{filter}} + F_{\text{out}})\, S_L - \frac{\mu V X}{Y_{X/L}} - k_{mL} V X \tag{5.4}$$

the variation of volume [l/h]:

$$\frac{dV}{dt} = F_{\text{in1}} + F_{\text{in2}} + F_{\text{in3}} - F_{\text{filter}} - F_{\text{out}} = 0 \tag{5.5}$$

By assuming constant volume (Eq. (5.5)) and substituting $D = F/V$ into Eqs. (5.2)–(5.4), an alternative formulation of Eqs. (5.2)–(5.4) is obtained:

$$\frac{dX}{dt} = (\mu - D_{\text{out}})\, X - k_d X \tag{5.6}$$

$$\frac{dS_Y}{dt} = -\frac{\mu X}{Y_{X/Y}} - (D_{\text{filter}} + D_{\text{out}})\, S_Y + D_{\text{in1}} S_{fY} - k_{mY} X \tag{5.7}$$

$$\frac{dS_L}{dt} = -\frac{\mu X}{Y_{X/L}} - (D_{\text{filter}} + D_{\text{out}})\, S_L + D_{\text{in2}} S_{fL} - k_{mL} X \tag{5.8}$$

where

- X— cell concentration.
- S_Y— substrate yeast extract-peptone concentration.
- S_{fY}—the feed yeast extract-peptone concentration.
- S_L— substrate lactic acid concentration.
- S_{fL}—the feed lactic acid concentration.
- μ— the specific growth rate.
- $Y_{X/Y}$— the cell growth yield coefficient on the substrate yeast extract-peptone.
- $Y_{X/L}$— the cell growth yield coefficient on the substrate lactic acid.
- k_d— the coefficient in the cell growth Eq. (5.2) to account for the natural death of cells.

- k_{mY} (or k_{mL}) —the coefficient in the substrate yeast extract-peptone (or lactic acid) consumption Eq. (5.3) (or Eq. (5.4)) to account for that part of the substrate used for cell survival.

- V— the bioreactor volume.

- $D_{\text{in1}} = F_{\text{in1}}/V$, $D_{\text{in2}} = F_{\text{in2}}/V$, $D_{\text{in3}} = F_{\text{in3}}/V$, $D_{\text{out}} = F_{\text{out}}/V$, $D_{\text{filter}} = F_{\text{filter}}/V$.

Note: the coefficients k_d and k_{mY} (or k_{mL}) are considered to be negligible in many industrial fermentations. Therefore they will be omitted in our case.

Due to the confidential factor concerned with the industry company involved, most variables and parameters are normalized appropriately in the following discussions. The cell concentration X is shown explicitly, instead of to be normalized, to highlight this process as the high concentration cultivation of cell mass. The dynamic model, which will be used in Chapters 5–7 of the thesis, is thus given in the following formulation:

$$\boxed{\frac{dX}{dt} = (\mu - D_{\text{out}})\, X} \tag{5.9}$$

$$\boxed{\frac{drS_Y}{dt} = -\frac{\mu X}{K_Y} - (D_{\text{filter}} + D_{\text{out}})\, rS_Y + D_{\text{in1}}} \tag{5.10}$$

$$\boxed{\frac{drS_L}{dt} = -\frac{\mu X}{K_L} - (D_{\text{filter}} + D_{\text{out}})\, rS_L + D_{\text{in2}}} \tag{5.11}$$

where

$rS_Y = S_Y/S_{fY}$— Normalized yeast extract-peptone concentration with respect to its feed concentration S_{fY}.

$rS_L = S_L/S_{fL}$— Normalized lactic acid concentration with respect to its feed concentration S_{fL}.

$K_L = Y_{X/L}S_{fL}$— Product of cell yield coefficient on lactic acid and lactic acid feed concentration.

$K_Y = Y_{X/Y}S_{fY}$— Product of cell yield coefficient on yeast extract-peptone and yeast extract-peptone feed concentration.

Other variables are understood according to the notations for Eqs. (5.6)–(5.8).

In this fermentation process yeast extract-peptone acts as growth-limiting substrate. Since the Monod model has been widely used to describe cell growth kinetics (see Section 4.3) and also based on the conclusion from our experimental results presented in Sections 4.5 and 4.6, we have used the Monod model for describing this industrial *Propionibacterium shermanii* fermentation process kinetics,

$$\boxed{\mu = \mu_m \frac{rS_Y}{rK_s + rS_Y}} \tag{5.12}$$

where μ_m represents the maximum specific cell growth rate and $rK_s = K_s/S_{fY}$ is the normalized saturation constant with respect to yeast extract-peptone feed concentration.

5.4 K_s's Influence on Steady State Behavior of Process

Due to the economical aspects of this commercial cell mass production process, the yeast extract-peptone is chosen to be used as growth-limiting substrate in order to decrease the cost of raw materials. However, this choice as a consequence puts extra difficulty on the operation of this process because yeast extract-peptone is a complex nitrogenous substrate which composition is undefined. As pointed out by Brock and Madigan (1991), an important disadvantage of using a complex medium is the loss of control over the precise nutrient specification of the medium. In our case, it is yet unknown which component of yeast extract-peptone limits the cell growth. To directly measure concentration of yeast extract-peptone is very difficult and the value of K_s (saturation constant in Monod model) can't be determined easily. Theoretically, we could analyse each component (i) of yeast extract-peptone and determine the corresponding K_{si} value, and then combine these individual K_{si} values to get K_s value which reflects the affinity of propionibacteria for yeast extract-peptone in a total sense. However, analysing each component of yeast extract-peptone would involve an enormous effort and would increase capital investments. To our knowledge, no study has been reported for propionibacteria fermentation using the complex nitrogenous substrate yeast extract-peptone as growth-limiting substrate. In other words, from the literature there is no any quantitative information available about K_s value corresponding to yeast extract-peptone as limiting substrate.

From the definition of K_s (see Section 4.3), K_s is inversely proportional to the affinity of microorganism for its substrate: a high K_s indicates weak binding of microorganism to the substrate; a low K_s indicates strong binding. In this section, simulation study results are presented which shows by a sensitivity analysis the effect of the K_s-value on the process behavior. This highlights the importance of the knowledge about K_s for good supervision and operation of the bioreactor.

5.4.1 Steady state behavior as function of K_s

From Eqs. (5.9–5.11), the steady state values of cell and substrate concentrations can be calculated as:

$$\frac{dX}{dt} = 0 \Rightarrow \mu = D_{\text{out}} \Rightarrow \boxed{rS_Y = \frac{rK_s\, D_{\text{out}}}{\mu_m - D_{\text{out}}}} \tag{5.13}$$

$$\frac{drS_Y}{dt} = 0 \Rightarrow \boxed{X = \frac{D_{\text{in1}} - (D_{\text{filter}} + D_{\text{out}})\, rS_Y}{D_{\text{out}}} K_Y} \tag{5.14}$$

$$\frac{drS_L}{dt} = 0 \Rightarrow \boxed{rS_L = \frac{D_{\text{in2}} - \frac{D_{\text{out}}\, X}{K_L}}{D_{\text{filter}} + D_{\text{out}}}} \tag{5.15}$$

The desired steady state values (relative values) as specified for this industrial process are listed in Table 5.1.

Table 5.1: Steady State Data

Dilution rate	D_{in1}	D_{in2}	D_{in3}	D_{filter}	D_{out}
$[\text{h}^{-1}]$	0.210	5.600	0.021	5.719	0.112
Concentration	X				
	29.429 [g/l]				
Parameters	μ_m	K_L	K_Y		
	0.141 $[\text{h}^{-1}]$	0.6 [g/l]	16 [g/l]		

For an increase in the value of K_s (rK_s), the concentration of the growth-limiting substrate YEP (rS_Y)† will increase, causing a large loss of the substrate YEP (which is much more expensive). Subsequently, the cell mass concentration

†In this study, the meaning of yeast extract-peptone concentration is in a total sense without considering each individual component of yeast extract-peptone.

(X) decreases, and thus the cell productivity ($P = D_{\text{out}}X$) decreases. The non-limiting substrate concentration (rS_L) will also increase, resulting in a large loss of substrate LA.

On the other hand, there are three inflow streams into this bioreactor and two outflow streams. By adjusting these flow rates, different special cases occur.

In the following simulation study, the value of the relative saturation constant (rK_s) ranges between 0.00017 and 0.0093 as concerned in our case.

Case 1. Adjust D_{in2} to keep rS_L constant at the desired lower level but not growth-limiting level, and adjust D_{filter} to keep the reactor volume V constant. D_{in1}, D_{in3} and D_{out} are assumed to be constant in this case.

As the reactor volume is constant, we have

$$D_{\text{filter}} = D_{\text{in2}} + D_{\text{in1}} + D_{\text{in3}} - D_{\text{out}} \tag{5.16}$$

By substituting Eq. (5.16) into Eq. (5.15), we get

$$D_{\text{in2}} = \frac{(D_{\text{in1}} + D_{\text{in3}})rS_L + \frac{D_{\text{out}}X}{K_L}}{1 - rS_L} \tag{5.17}$$

By substituting Eq. (5.16) and Eq. (5.17) into Eq. (5.14), we get

$$X = \frac{D_{\text{in1}} - \frac{D_{\text{in1}}+D_{\text{in3}}}{1-rS_L}rS_Y}{\frac{D_{\text{out}}}{K_Y} + \frac{D_{\text{out}}rS_Y}{K_L(1-rS_L)}} \tag{5.18}$$

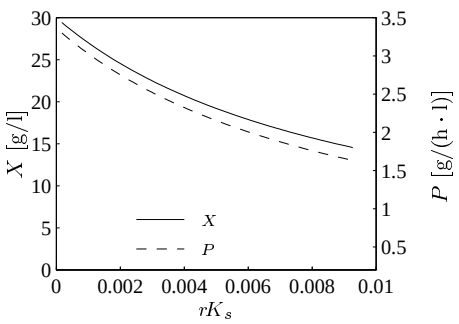
As rS_Y varies with rK_s, see Eq. (5.13), X is a function of only rK_s since D_{in1}, D_{in3} and D_{out} are constant.

The steady state behavior of X, rS_Y, D_{in2} and D_{filter} as functions of rK_s are shown in Figure 5.2. As rS_Y increases with rK_s (see Figure 5.2(b)), X decreases sharply (see Figure 5.2(a)) and then results in the decrease in the cell productivity. Although D_{filter} decreases, much more YEP substrate is lost due to so higher rS_Y (see the dashdot curve in Figure 5.2(c)). Since rS_L is kept constant by decreasing D_{in2}, no LA substrate is lost.

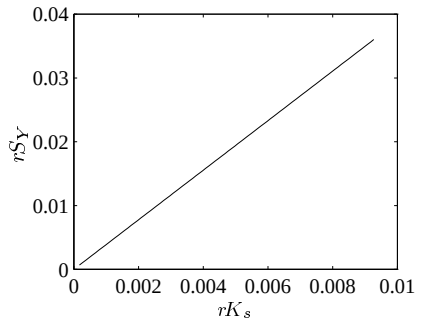
Case 2. Adjust D_{in1} in order to keep X constant (or unaffected by rK_s), and also (as in Case 1) adjust D_{in2} to keep rS_L constant and adjust D_{filter} to keep V constant. D_{in3} and D_{out} are assumed constant here.

rS_Y, D_{filter} and D_{in2} vary with rK_S, respectively, as given by Eq. (5.13), Eq. (5.16) and Eq. (5.17). From Eq. (5.14), we have

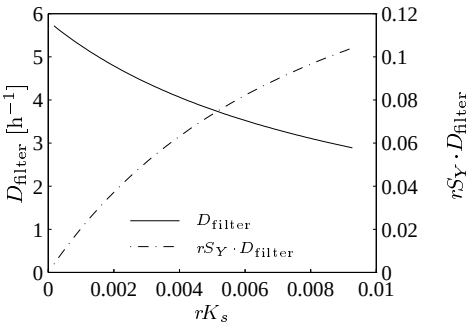
$$D_{\text{in1}} = (D_{\text{filter}} + D_{\text{out}})rS_Y + \frac{D_{\text{out}}X}{K_Y} \tag{5.19}$$



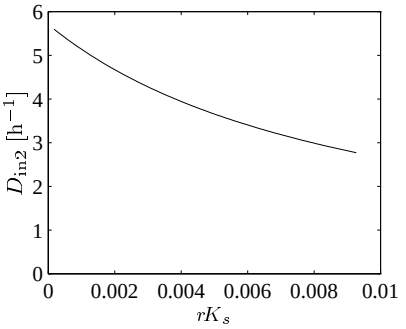
(a) Effect of rK_s on X



(b) Effect of rK_s on rS_Y



(c) Effect of rK_s on D_{filter}



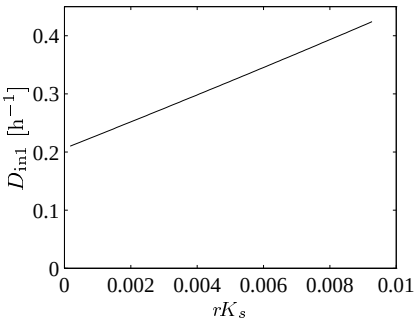
(d) Effect of rK_s on D_{in2}

Figure 5.2: Steady state behavior of X, rS_Y, D_{in2}, and D_{filter} as functions of rK_s, respectively. Other process variables rS_L, D_{in1}, D_{in3}, D_{out} and V are kept constant in this case study.

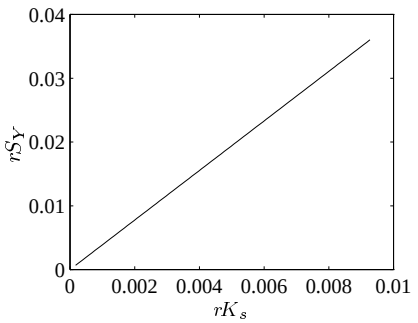
By substituting Eq. (5.16) and Eq. (5.17) into Eq. (5.19), we get an alternative expression of D_{in1}:

$$D_{\text{in1}} = \frac{\frac{D_{\text{out}}X}{K_Y} + (D_{\text{in3}} + \frac{D_{\text{out}}X}{K_L})\frac{rS_Y}{1-rS_L}}{1 - \frac{rS_Y}{1-rS_L}} \tag{5.20}$$

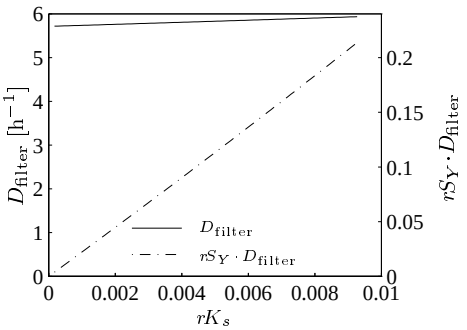
When D_{out} is unchanged, rS_Y is a linear function of rK_s (recall Eq. (5.13)). Here $1 - \frac{rS_Y}{1-rS_L} \approx 1$ because yeast extract-peptone is limiting substrate and then its concentration S_Y is very low especially compared to the yeast extract-peptone feed concentration S_{fY}, hence $rS_Y = S_Y/S_{fY} \approx 0$. We thus concluded that D_{in1} is also a linear function of rK_s when D_{in3} and D_{out} are constant.



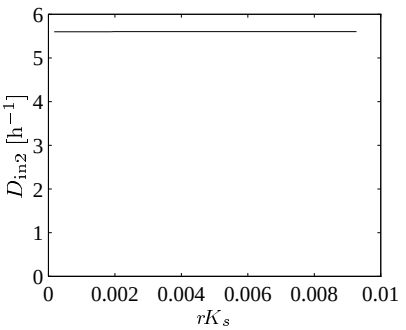
(a) Effect of rK_s on D_{in1}



(b) Effect of rK_s on rS_Y



(c) Effect of rK_s on D_{filter}



(d) Effect of rK_s on D_{in2}

Figure 5.3: Steady state behavior of D_{in1}, rS_Y, D_{in2}, and D_{filter} as functions of rK_s, respectively. Other process variables X, rS_L, D_{in3}, D_{out} and V are kept constant in this case study.

The simulation results on steady state behavior, as shown in Figure 5.3, indicate that rK_s has a large effect on D_{in1} and rS_Y (see Figure 5.3(a–b), whereas D_{in2} and D_{filter} are relatively insensitive to changes in rK_s in this case (see Figure 5.3(c–d). As X is kept constant with rK_s and D_{out} is unchanged, the cell productivity is not decreased. Since rS_L is constant and D_{filter} varies only slightly, the amount of lost LA substrate is negligible. However, still much more YEP substrate is lost due to so higher rS_Y similar as in Case 1.

Case 3. Adjust D_{out} in order to keep rS_Y constant (or unaffected by rK_s), also adjust D_{in1} in order to keep X constant as in Case 2, and adjust D_{in2} to keep rS_L constant and adjust D_{filter} to keep V constant, respectively, as in Case 1.

From Eq. (5.13), we get

$$D_{\text{out}} = \mu_m \frac{rS_Y}{rK_s + rS_Y} \tag{5.21}$$

D_{filter}, D_{in2} and D_{in1} vary with rK_S, respectively, as given in Eq. (5.16), Eq. (5.17) and Eq. (5.20).

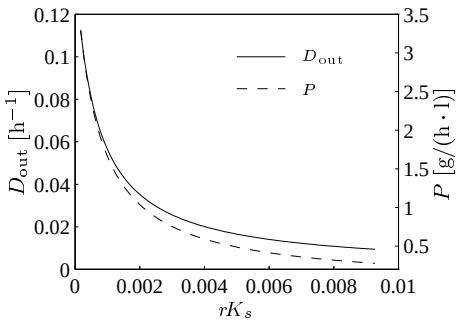
From the simulation results on steady state behavior as shown in Figure 5.4, substrates are saved without losing since both rS_Y and rS_L are kept constant and flow rates are decreased. However, we find that D_{out} decreases sharply with increasing rK_s and then the cell productivity is heavily decreased (see Figure 5.4(a)). In practice, there is almost no real production as all flow rates have to be decreased in order to compensate the influence of high K_s-value on concentrations of cell mass and substrates.

5.4.2 Steady state behavior as function of flow rate

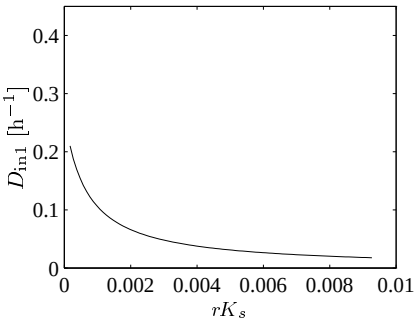
Steady state relationship between cell concentration X and cell production rate D_{out}

As shown in Figure 5.5, when D_{out} increases the cell concentration X decreases and substrate concentrations rS_Y and rS_L increase. Changes in D_{out} is balanced by adjusting D_{filter} in order to keep V constant. If D_{out} reaches a critical value $D_{\text{out}_{\text{max}}}$ which can be calculated from Eq. (5.14) (see Appendix),

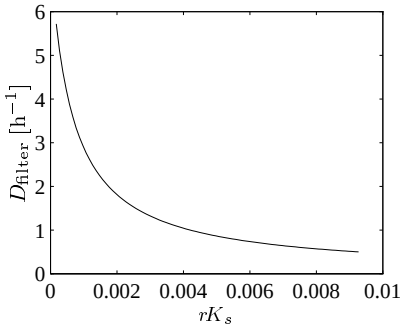
$$D_{\text{out}_{\text{max}}} = \frac{\mu_m D_{\text{in1}}}{rK_s(D_{\text{in1}} + D_{\text{in2}} + D_{\text{in3}}) + D_{\text{in1}}} \tag{5.22}$$



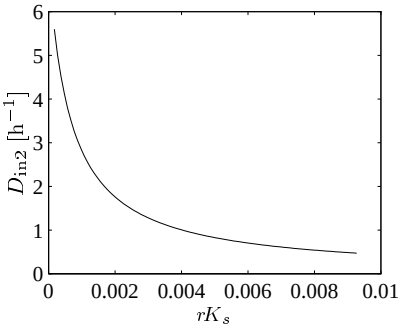
(a) Effect of rK_s on D_{out}



(b) Effect of rK_s on D_{in1}

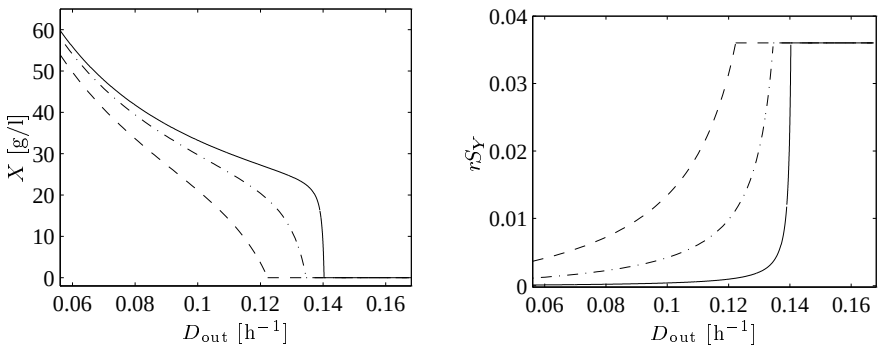


(c) Effect of rK_s on D_{filter}



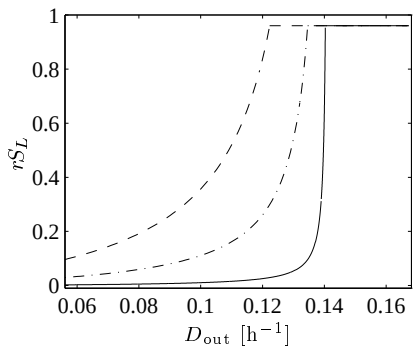
(d) Effect of rK_s on D_{in2}

Figure 5.4: Steady state behavior of D_{out}, D_{in1}, D_{in2}, and D_{filter} as functions of rK_s, respectively. Other process variables X, rS_Y, rS_L, D_{in3} and V are kept constant in this case study.

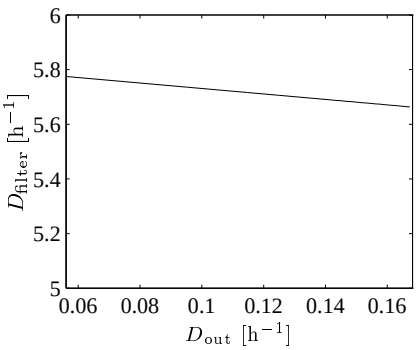


(a) Effect of D_{out} on X

(b) Effect of D_{out} on rS_Y



(c) Effect of D_{out} on rS_L



(d) Effect of D_{out} on D_{filter}

line type	rK_s	$D_{\text{out}_{\text{max}}}$ [h^{-1}]
solid curves	.00017	.140
dash-dot curves	.0017	.135
dashed curves	.0055	.122

Figure 5.5: Steady state values of X, rS_L and rS_Y as functions of D_{out} with other flow rates kept constant, except D_{filter} which is adjusted to keep V constant. The three curves correspond to different rK_s values.

the cell can not grow fast enough to keep up with its outflow. The culture is thus washed out of the reactor and then rS_L and rS_Y rise to their maximum values, respectively,

$$rS_{Y_{\max}} = \frac{D_{\text{in1}}}{D_{\text{in1}} + D_{\text{in2}} + D_{\text{in3}}} \tag{5.23}$$

$$rS_{L_{\max}} = \frac{D_{\text{in2}}}{D_{\text{in1}} + D_{\text{in2}} + D_{\text{in3}}} \tag{5.24}$$

The critical value $D_{\text{out}_{\max}}$ depends on the saturation constant rK_s (see Eq. (5.22)). The higher the saturation constant rK_s is, the lower the critical value $D_{\text{out}_{\max}}$ becomes, i.e. washout more easily occurs when rK_s's value is higher.

In this case, flow rates D_{in1}, D_{in2} and D_{in3} are constant.

Steady state relationship between cell concentration X and growth-limiting substrate YEP feed flow rate D_{in1}

When flow rates D_{in2}, D_{in3} and D_{out} are constant, X is proportional to D_{in1} as shown in Figure 5.6(a). The changes in D_{in1} is compensated by adjusting D_{filter} to keep V constant. rS_Y does not vary with D_{in1}. rS_L decreases with increasing D_{in1} and then this gives an upper bound to D_{in1} (see Figure 5.6(c)), i.e. D_{in1} should be below $D_{\text{in1}_{\max}}$ which can be calculated from Eq. (5.15) (see Appendix),

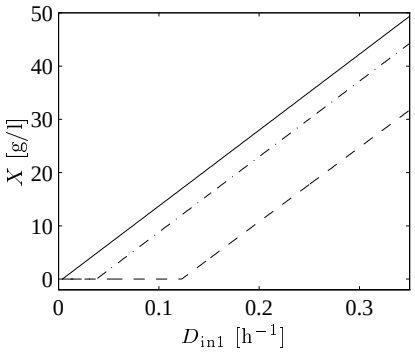
$$D_{\text{in1}_{\max}} = \frac{D_{\text{in2}}K_L + rS_Y K_Y (D_{\text{in2}} + D_{\text{in3}})}{K_Y(1 - rS_Y)} \tag{5.25}$$

otherwise $rS_L = 0$.

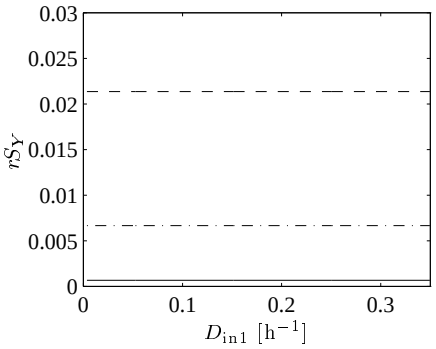
On the other hand, D_{in1} should be above a critical value $D_{\text{in1}_{\min}}$, that is, in order to have $X > 0$, we should have

$$D_{\text{in1}} > D_{\text{in1}_{\min}} = (D_{\text{in2}} + D_{\text{in3}})\frac{rS_Y}{1 - rS_Y} \tag{5.26}$$

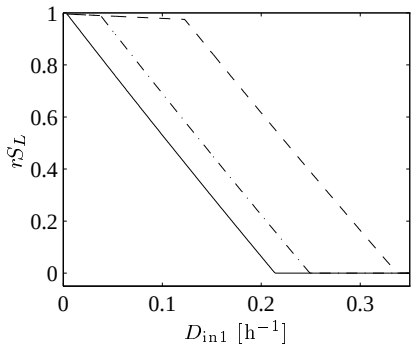
otherwise at very low flow rates, a large fraction of cells may die from starvation since the limiting substrate is not being added fast enough to permit maintenance of cell growth. $D_{\text{in1}_{\min}}$ also depends on rK_s. When facing higher rK_s, more limiting substrates should be added to maintain cell growth.



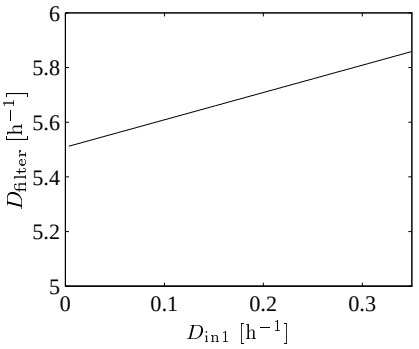
(a) Effect of D_{in1} on X



(b) Effect of D_{in1} on rS_Y



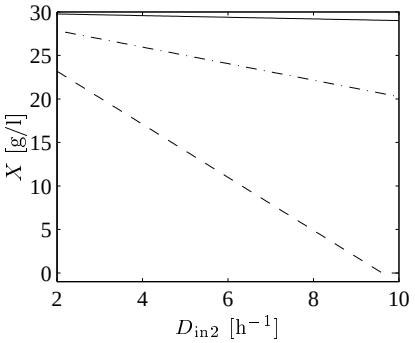
(c) Effect of D_{in1} on rS_L



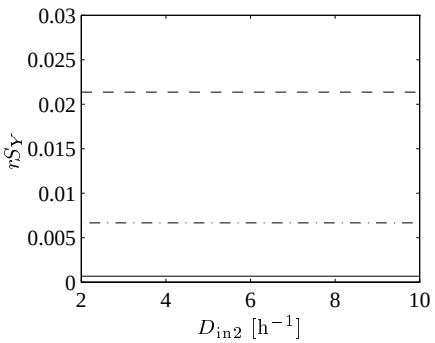
(d) Effect of D_{in1} on D_{filter}

line type	rK_s	$D_{\text{in1}_{\text{min}}}$ [h^{-1}]	$D_{\text{in1}_{\text{max}}}$ [h^{-1}]
solid curves	.00017	.0037	.214
dash-dot curves	.0017	.037	.249
dashed curves	.0055	.123	.337

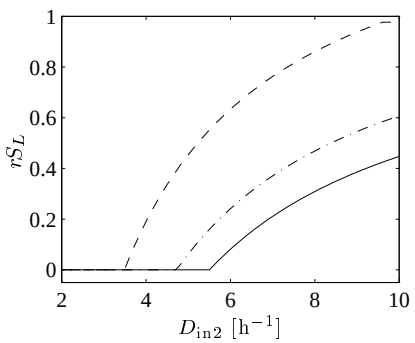
Figure 5.6: Steady state values of X, rS_L and rS_Y as functions of D_{in1} with other flow rates at constant, except D_{filter} which is adjusted to keep V constant. The three curves correspond to different rK_s values.



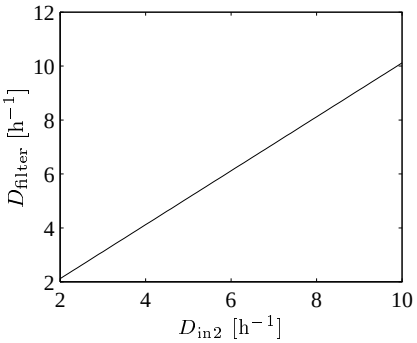
(a) Effect of D_{in2} on X



(b) Effect of D_{in2} on rS_Y



(c) Effect of D_{in2} on rS_L



(d) Effect of D_{in2} on D_{filter}

line type	rK_s	$D_{\text{in2}_{\text{min}}}$ [h^{-1}]	$D_{\text{in2}_{\text{max}}}$ [h^{-1}]
solid curves	.00017	5.502	315.174
dash-dot curves	.0017	4.724	31.310
dashed curves	.0055	3.486	9.604

Figure 5.7: Steady state values of X, rS_L and rS_Y as functions of D_{in2} with other flow rates kept constant, except D_{filter} which is adjusted to keep V constant. The three curves correspond to different rK_s values.

Steady state relationship between cell concentration X and substrate LA feed flow rate D_{in2}

In this case flow rates D_{in1}, D_{in3} and D_{out} are constant, and the growth-limiting substrate YEP concentration rS_Y doesn't vary with D_{in2} since rS_Y only depends on D_{out} which is unchanged in this case. Of course rS_L increases with increasing D_{in2}, and this gives rise to a lower bound to D_{in2}, i.e. D_{in2} should be above $D_{\text{in2}_{\min}}$, which can be calculated from Eq. (5.15) (see Appendix),

$$D_{\text{in2}_{\min}} = [D_{\text{in1}} - (D_{\text{in1}} + D_{\text{in3}})rS_Y] \frac{K_Y}{K_L + rS_Y K_Y} \tag{5.27}$$

otherwise $rS_L = 0$.

At very low rK_s, X is insensitive to the changes in D_{in2} as shown in Figure 5.7(a). However at higher rK_s, X will decrease with increasing D_{in2}. Therefore, there also exists a upper bound to D_{in2},

$$D_{\text{in2}_{\max}} = \frac{D_{\text{in1}}}{rS_Y} - (D_{\text{in1}} + D_{\text{in3}}) \tag{5.28}$$

D_{in2} should be below $D_{\text{in2}_{\max}}$ in order to avoid washout. $D_{\text{in2}_{\max}}$ will decreases as rK_s increases, i.e. with higher rK_s value, washout would more easily happen. This phenomenon is explained as follow. The changes in D_{in2} are balanced by adjusting D_{filter} in order to keep V constant. Since D_{in2} and D_{filter} are both large flow rates, and corresponding to very higher D_{in2} we should have much higher D_{filter} to keep V unchanged. And this would result in much more substrates washed out of reactor by filtration before cells are able to consume them for cell synthesis, in other words, less substrates in the reactor are available for cell growth. On the other hand, the filter is easily getting to be clotted when facing large flow rate. If that happens, feeding pumps have to be turned off and then no feed streams go into the reactor which results in a failure of the reactor.

5.5 Concluding Remarks

Generally, in this continuous bioreactor with multiple substrate streams the cell concentration is more sensitive to changes in D_{out}, which is very different from a general biochemical CSTR with single substrate stream where the cell concentration almost remains constant throughout most of the range of the dilution rates (except close to the $D_{\text{out}_{\max}}$). The growth-limiting substrate concentration

rS_Y only depends on the cell production rate D_{out} similar as in a general CSTR. In addition, X is also sensitive to changes in D_{in1} but rather insensitive to the changes in D_{in2} when facing lower rK_s. rS_L is sensitive to changes in feed flow rates D_{in1} and D_{in2} but almost remains constant throughout most of the range of D_{out}.

Specially, regarding to the K_s's influence on the process behavior, we concluded that when K_s's value is higher, there would exist the following problems:

(1). A high value of K_s means that the limiting substrate YEP concentration rS_Y should be high in order to attain the desired cell specific growth rate μ (recall Eq. (5.12)), and thus the designed high cell concentration can not be reached under the given operating conditions (flow rates unchanged). In order to obtain the desired high concentration of cell mass, more limiting substrate YEP needs to be added into the reactor (increasing D_{in1}). On the other hand, more limiting substrate YEP is lost as a result of high rS_Y. We could save substrates at the price of low cell productivity.

(2) The allowed variation ranges for flow rates are getting narrow. In other words, from the control point of view, the problem associated with manipulated input constraints becomes considerable.

(3) Washout occurs more easily, and results in a failure of the reactor.

Nomenclature

LA	lactic acid	
YEP	mixture of yeast extract and peptone	
D_{in1}	yeast extract-peptone influent flow rate	$[\text{h}^{-1}]$
D_{in2}	lactic acid influent flow rate	$[\text{h}^{-1}]$
D_{in3}	base influent flow rate	$[\text{h}^{-1}]$
D_{filter}	filtrate flow rate	$[\text{h}^{-1}]$
D_{out}	product effluent flow rate	$[\text{h}^{-1}]$
K_s	saturation constant in Monod model	[g/l]
$P = D_{\text{out}}X$	cell productivity	[g cells/(h · l)]
S	substrate concentration	[g/l]
S_L	substrate lactic acid concentration	[g/l]
S_{fL}	the feed lactic acid concentration	[g/l]
$K_L = Y_{X/L}S_{fL}$	product of cell yield coefficient on lactic acid and lactic acid feed concentration	[g/l]

$rS_L = S_L/S_{fL}$	normalized LA concentration with respect to its feed concentration S_{fL}	
S_Y	substrate YEP concentration	[g/l]
S_{fY}	the feed YEP concentration	[g/l]
$K_Y = Y_{X/Y}S_{fY}$	product of cell yield coefficient on YEP and YEP feed concentration	[g/l]
$rS_Y = S_Y/S_{fY}$	normalized YEP concentration with respect to its feed concentration S_{fY}	
$rK_s = K_s/S_{fY}$	normalized saturation constant with respect to YEP feed concentration	
V	the bioreactor volume	[l]
X	cell concentration	[g/l]
$Y_{X/L}$	cell growth yield coefficient on LA	[g cells/ g LA]
$Y_{X/Y}$	cell growth yield coefficient on YEP	[g cells/ g YEP]

Greek Symbols

μ	specific growth rate	$[h^{-1}]$
μ_m	maximum specific growth rate	$[h^{-1}]$

References

Brock, T. D. and Madigan, M. T. (1991). *Biology of Microorganisms*, 6th edn, Prentice-Hall, Englewood Cliffs.

Crueger, W. and Crueger, A. (1990). *Biotechnology: A Textbook of Industrial Microbiology*, second edn, Sinauer Associates, Inc., Sunderland, MA 01375, USA.

Appendix. Derivation of bounds on flow rates

Physically, all concentrations of cell mass and substrates in the fermenter-filter system must be positive, and this requirement puts bounds on flow rates, i.e. the flow rates should be within their allowed variation ranges to maintain the reactor under rational operation.

1. Upper bound of D_{out}

From Eq. (5.14), we get

$$X > 0 \Longleftrightarrow D_{\text{in1}} > (D_{\text{filter}} + D_{\text{out}})rS_Y \tag{5.29}$$

As rS_Y is only a function of D_{out} and V is held constant by adjusting D_{filter}, by substituting Eqs. (5.13) and (5.16) into Eq. (5.29), we derive

$$D_{\text{out}} < \frac{\mu_m D_{\text{in1}}}{rK_s(D_{\text{in1}} + D_{\text{in2}} + D_{\text{in3}}) + D_{\text{in1}}} \tag{5.30}$$

which gives

$$X > 0,\ rS_Y > 0,\ \text{and}\ rS_L > 0$$

If D_{out} reaches the critical value $D_{\text{out}_{\max}}$, washout occurs. Substitution of $D_{\text{out}_{\max}}$ (Eq. (5.22)) for D_{out} in Eqs. (5.13)–(5.15) will result in

$$X = 0$$

$$rS_Y = rS_{Y_{\max}} = \frac{D_{\text{in1}}}{D_{\text{in1}} + D_{\text{in2}} + D_{\text{in3}}}$$

$$rS_L = rS_{L_{\max}} = \frac{D_{\text{in2}}}{D_{\text{in1}} + D_{\text{in2}} + D_{\text{in3}}}$$

2. Lower and upper bounds of D_{in1}

Flow rates D_{in2}, D_{in3}, and D_{out} are assumed to be unchanged, and rS_Y is thus constant as it only depends on D_{out}. V is constant by adjusting D_{filter}. Substituting Eq. (5.16) into Eq. (5.29) yields

$$D_{\text{in1}_{\min}} = (D_{\text{in2}} + D_{\text{in3}})\frac{rS_Y}{1 - rS_Y} \tag{5.31}$$

From Eq. (5.15), we have

$$rS_L > 0 \Longleftrightarrow D_{\text{in2}} > \frac{D_{out}X}{K_L} \tag{5.32}$$

As X is the function of D_{in1}, substituting Eq. (5.16) into Eq. (5.14) yields

$$X = [D_{\text{in1}}(1 - rS_Y) - (D_{\text{in2}} + D_{\text{in3}})rS_Y]\,\frac{K_Y}{D_{\text{out}}} \tag{5.33}$$

and by substituting Eq. (5.33) into Eq. (5.32), the upper bound of D_{in1} is derived

$$D_{\text{in1}_{\max}} = \frac{D_{\text{in2}}\frac{K_L}{K_Y} + (D_{\text{in2}} + D_{\text{in3}})rS_Y}{1 - rS_Y} \tag{5.34}$$

Therefore we should have $D_{\text{in1}_{\min}} < D_{\text{in1}} < D_{\text{in1}_{\max}}$ so that $X > 0$ and $rS_L > 0$.

3. Lower and upper bounds of D_{in2}

We consider here only changes in D_{in2} with other flow rates constant except D_{filter} is adjusted to keep V unchanged. rS_Y is constant because of constant D_{out}.

The Eq. (5.32) can be rewritten by substituting Eq. (5.33) for X,

$$D_{\text{in2}} > [D_{\text{in1}}(1 - rS_Y) - (D_{\text{in2}} + D_{\text{in3}})rS_Y] \frac{K_Y}{K_L} \tag{5.35}$$

This gives the lower bound to D_{in2}

$$D_{\text{in2}_{\min}} = [D_{\text{in1}}(1 - rS_Y) - D_{\text{in3}}rS_Y] \frac{K_Y}{K_L + rS_Y K_Y} \tag{5.36}$$

Similarly, by substituting Eq. (5.16) for D_{filter} and rewriting Eq. (5.29) as

$$D_{\text{in1}} > (D_{\text{in1}} + D_{\text{in2}} + D_{\text{in3}})rS_Y \tag{5.37}$$

the upper bound of D_{in2} is given by

$$D_{\text{in2}_{\max}} = \frac{D_{\text{in1}}}{rS_Y} - (D_{\text{in1}} + D_{\text{in3}}) \tag{5.38}$$

So in order to have $X > 0$ and $rS_L > 0$ we must keep D_{in2} within its allowed range, i.e. $D_{\text{in2}_{\min}} < D_{\text{in2}} < D_{\text{in2}_{\max}}$.

Chapter 6

High-concentration Cultivation of Propionibacteria in Continuous Bioreactor with Cross-flow Filtration. Part II: Estimation

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6.1 Introduction

The knowledge of the process state variables is important in any type of control system. Unfortunately, on-line measurement of the important state variables in biotechnical processes, particularly cell concentration, remains a difficult problem. However a number of secondary or environmental variables can be on-line measured conventionally, such as pH, and CO_2 and O_2 in the exhaust gas.

A modeling methodology first presented by Stephanopoulos and San (1984), based on species balances, provides relations between the environmental and important state variables. Using such a model, the important state variables can be estimated in principle from more easily accessible on-line measurements. Ramseier et al. (1993) used the same approach as proposed by Stephanopoulos and San (1984), but modified the model to match the process characteristics of the particular experimental fermentation system. Specially, they developed a nonlinear model that provides relation between cell mass and two environmental variables, i.e. the base addition rate and the CO_2 offgas concentration which are measured on-line and periodically. They designed a new adaptive cell estimator by incorporating as its basis such an underlying nonlinear model so as to utilize the best possible a priori process knowledge.

The objective of this work is to use the on-line measurable environmental variables to estimate the unmeasured state variables in propionibacteria fermentation process. A dynamic model is developed in Section 6.2 which provides interrelationship between state variables and measured environmental variables, and a nonlinear estimator is described in Section 6.3. Section 6.4 shows simulation results of on-line estimation of concentrations of cell mass and substrates.

6.2 Development of Cell Estimation Model

As mentioned above, in order to use environmental variables to estimate the unmeasured state variables, we first have to model their interrelationship.

In propionibacteria fermentation, CO_2 is the only gas produced, so the on-line measurement of CO_2 evolution rate is simple since we do not need to analyze gas compositions. Furthermore, the CO_2 production rate is directly related to the lactic acid consumption rate from the stoichiometry (recall Eq. (4.5)), and is also related to the cell growth rate because of "growth associated" product formation by *Propionibacterium shermanii* (Tyree et al., 1991). On the basis of the material

balance, we then have the following equation to describe CO_2 accumulation rate,

$$\boxed{\frac{dC}{dt} = \frac{\mu X}{Y_{X/CO_2}} - (D_{\text{filter}} + D_{\text{out}})C - D_{\text{co}_2}\rho_{\text{co}_2}} \tag{6.1}$$

where

C— dissolved carbon dioxide concentration in the medium.

D_{co_2}— carbon dioxide evolution rate in the exhaust gas.

ρ_{co_2}— carbon dioxide gas density.

Y_{X/co_2}— the cell yield coefficient on the carbon dioxide.

The other variables are defined in Section 5.3; see Eqs. (5.6)–(5.8).

For the measurement of the dissolved carbon dioxide concentration in culture medium, reliable sensors have been developed by Shoda and Ishikawa (1981), Spinnler et al. (1987) and Dahod (1993). Therefore the information on cell growth rate can be inferred from the CO_2 measurement according to the above Eq. (6.1).

In addition, we have another available on-line measurement of a secondary variable, namely the base addition rate D_{in3} which is adjusted in order to keep the pH constant. By analyzing previous experimental data provided by the Biotechnology group in SINTEF Applied Chemistry, we found that the base addition rate seems to be related to the cell growth rate. We now want to develop a mathematical model for this relationship. Based on two assumptions:

- pH changes in fermenter are caused only by the organic acids produced from yeast extract-peptone.
- No accumulation of base in the fermenter (because the dynamics of compensating pH change by adding base solution is very fast compared to the cell growth dynamics).

we have the dynamic equation for base consumption,

$$\frac{dB}{dt} = -\frac{1}{Y_{X/base}}\mu X + D_{\text{in3}}B_f = 0 \tag{6.2}$$

Then it is now possible to on-line estimate the cell growth rate as a function of the directly on-line measurable variable D_{in3} as follows

$$\boxed{\mu X = Y_{X/base}D_{\text{in3}}B_f = K_B D_{\text{in3}}} \tag{6.3}$$

where

$Y_{X/base}$— the cell yield coefficient on base solution.

B_f— the concentration of feed base solution.

Therefore, Eqs. (6.1) and (6.3) furnish the foundations for the estimation of cell mass and substrate concentrations.

6.3 Discrete-time Nonlinear Filtering Problem

By adding Eq. (6.1) for the dynamics of dissolved CO_2 to the state equations (5.9)–(5.11) and rewriting them in vector form:

$$\frac{dx}{dt} = f(x) \tag{6.4}$$

where

$$x = \begin{bmatrix} X \\ rS_Y \\ rS_L \\ C \end{bmatrix}, \quad f(x) = \begin{bmatrix} (\mu - D_{\text{out}})X \\ -\frac{\mu X}{K_Y} - (D_{\text{filter}} + D_{\text{out}})\, rS_Y + D_{\text{in1}} \\ -\frac{\mu X}{K_L} - (D_{\text{filter}} + D_{\text{out}})\, rS_L + D_{\text{in2}} \\ \frac{\mu X}{Y_{X/CO_2}} - (D_{\text{filter}} + D_{\text{out}})C - D_{\text{co}_2}\rho_{\text{co}_2} \end{bmatrix}$$

As usual, Monod model is used to describe the cell growth kinetics in this fermentation process,

$$\mu = \mu_m \frac{rS_Y}{rK_s + rS_Y}$$

Measurement equation:

$$y = g(x) = \begin{bmatrix} \mu X \\ C \end{bmatrix} \tag{6.5}$$

Discretization. Let $x(t) = x_k$ and $x(t+T) = x_{k+1}$, and apply the Euler approximation $\frac{dx}{dt} \approx \frac{x(k+1)-x(k)}{T}$ for the time derivative in Eq. (6.4) to obtain the discrete-time form:

$$x_{k+1} = x_k + Tf(x_k) = F(x_k) \tag{6.6}$$

$$y_k = g(x_k) \tag{6.7}$$

By the incorporation of process noise w_k and measurement noise v_k into state and measurement equations, we have the discrete-time nonlinear system as follows:

$$x_{k+1} = F(x_k) + w_k \tag{6.8}$$

$$y_k = g(x_k) + v_k \tag{6.9}$$

Here we suppose that $[v_k]$ and $[w_k]$ are independent, zero mean, Gaussian white processes with

$$E[v_k v_k^T] = R_k \delta_{kl}, \quad E[w_k w_k^T] = Q_k \delta_{kl} \tag{6.10}$$

Linearization. We introduce the following notations:

$\hat{x}_{k|k-1}$ — the predicted estimate of x_k using measurements up till time $k-1$.

$\hat{x}_k$ — the filtered estimate of x_k using measurements up till time k.

Expanding the nonlinear functions $F(x_k)$ and $g(x_k)$ in Taylor series around the estimates $\hat{x}_k$ and $\hat{x}_{k|k-1}$, and neglecting higher order terms yields the approximate linear system:

$$x_{k+1} = A_k x_k + B_k + w_k \tag{6.11}$$

$$y_k = H_k^T x_k + D_k + v_k \tag{6.12}$$

where A_k, B_k, $H_{k|k-1}$ and D_k are calculated on-line from following equations:

$$A_k = \left.\frac{\partial F}{\partial x}\right|_{x=\hat{x}_k} = I + T \left.\frac{\partial f}{\partial x}\right|_{x=\hat{x}_k} \tag{6.13}$$

$$\frac{\partial f}{\partial x} = \begin{bmatrix} \mu - D_{\text{out}} & \frac{d\mu}{drS_Y} X & 0 & 0 \\ -\frac{\mu}{K_Y} & -\frac{d\mu}{drS_Y}\frac{X}{K_Y} - D_{\text{filter}} - D_{\text{out}} & 0 & 0 \\ -\frac{\mu}{K_L} & -\frac{d\mu}{drS_Y}\frac{X}{K_L} & -D_{\text{filter}} - D_{\text{out}} & 0 \\ -\frac{\mu}{Y_{X/CO_2}} & \frac{d\mu}{drS_Y}\frac{X}{Y_{X/CO_2}} & 0 & -D_{\text{filter}} - D_{\text{out}} \end{bmatrix}$$

$$\begin{aligned} B_k &= F(\hat{x}_k) - A_k \hat{x}_k = T\left(f(\hat{x}_k) - \left.\frac{\partial f}{\partial x}\right|_{x=\hat{x}_k} \hat{x}_k\right) \\ &= T \begin{bmatrix} -\frac{d\mu}{drS_Y} X r S_Y \\ D_{\text{in1}} + \frac{d\mu}{drS_Y}\frac{X r S_Y}{K_Y} \\ D_{\text{in2}} + \frac{d\mu}{drS_Y}\frac{X r S_Y}{K_L} \\ -D_{\text{co}_2}\rho_{\text{co}_2} - \frac{d\mu}{drS_Y}\frac{X r S_Y}{Y_{X/CO_2}} \end{bmatrix}_{x=\hat{x}_k} \end{aligned} \tag{6.14}$$

$$H_k^T = \left.\frac{\partial g}{\partial x}\right|_{x=\hat{x}_{k|k-1}} = \begin{bmatrix} \mu & \frac{d\mu}{drS_Y} X & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}_{x=\hat{x}_{k|k-1}} \tag{6.15}$$

$$D_k = g(\hat{x}_{k|k-1}) - H_k^T \hat{x}_{k|k-1} = \begin{bmatrix} -\frac{d\mu}{drS_Y} X r S_Y \\ 0 \end{bmatrix}_{x=\hat{x}_{k|k-1}} \tag{6.16}$$

and as $\mu = \mu_m \frac{rS_Y}{rK_s + rS_Y}$, we have

$$\frac{d\mu}{drS_Y} = \frac{\mu_m rK_s}{(rK_s + rS_Y)^2}$$

Successful application of extended Kalman filter (EKF) for state estimation in fermentation processes has been reported in the literature (see Section 2.2.2 for literature review on EKF). The EKF structure is well suited for fermentation systems because it is capable of dealing with nonlinear process and providing combined process state and model parameter estimation. Therefore we use EKF for on-line state estimation in this propionibacteria fermentation process.

Based on the above model, the corresponding EKF equations are given as follows:

Time update:

$$\hat{x}_{k|k-1} = F(\hat{x}_{k-1}) \tag{6.17}$$

$$P_{k|k-1} = A_{k-1} P_{k-1} A_{k-1}^T + Q_{k-1} \tag{6.18}$$

Observation update:

$$L_k = P_{k|k-1} H_k (H_k^T P_{k|k-1} H_k + R_k)^{-1} \tag{6.19}$$

$$\hat{x}_k = \hat{x}_{k|k-1} + L_k \left(y_k - g(\hat{x}_{k|k-1}) \right) \tag{6.20}$$

$$P_k = (I - L_k H_k^T) P_{k|k-1} \tag{6.21}$$

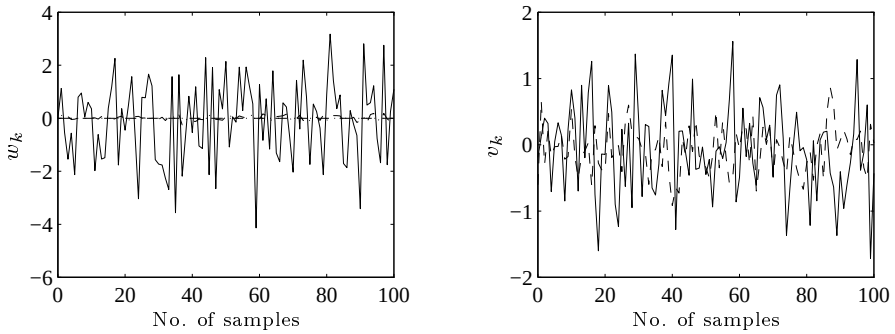
Initial condition:

$P_{0|-1} = 0$, $\hat{x}_{0|-1} = x_0 = [\, X_0 \quad rS_{Y_0} \quad rS_{L_0} \quad C_0 \,]^T$, and $L_0 = 0$ (calculated from Eq. (6.19)), $\hat{x}_0 = \hat{x}_{0|-1}$ (from Eq. (6.20)), $P_0 = 0$ (from Eq. (6.21)).

6.4 Simulation Results and Discussion

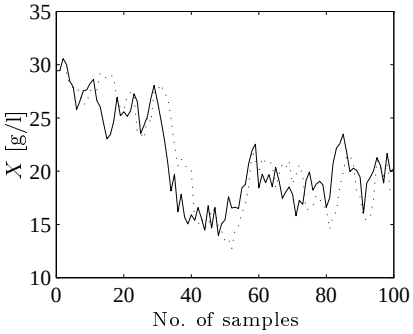
The objective of numerical study is to examine the ability of the estimator to produce the on-line estimation of process state variables from the measurements of environmental variables. The parameters and initial state values used in the simulation study are given in Table 6.1.

The discrete step size T in Euler approximation is chosen to be 0.05 hour (3 minutes), which is at least a hundred times smaller than the fermentation time constant (cell doubling time is about 6 hours in our case), so that the Euler approximation is reasonable.

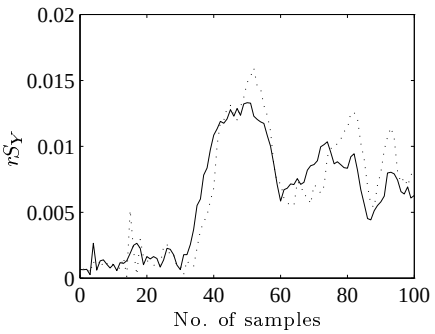


(a) Process noise

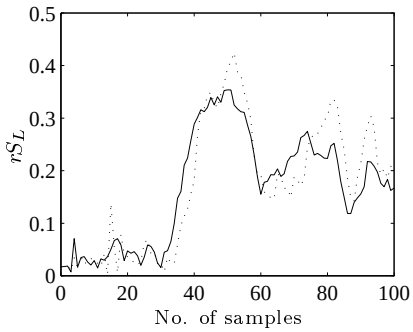
(b) Measurement noise



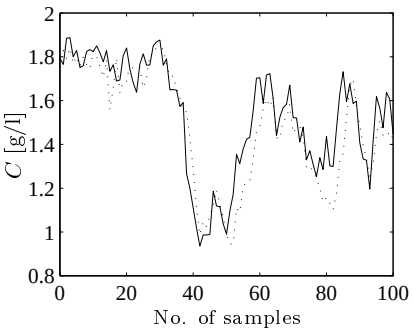
(c) Cell conc. estimation



(d) YEP conc. estimation



(e) LA conc. estimation



(f) CO_2 conc. estimation

Figure 6.1: Estimation results by applying EKF. Values of parameters and initial conditions are from Table 6.1. In subfigures (c)–(f), solid curves denote "true" values; dotted curves denote estimated values.

Table 6.1: Parameters and initial conditions for applying EKF

Dilution rate	D_{in1}	D_{in2}	D_{in3}	D_{filter}	D_{out}	D_{co_2}
$[\text{h}^{-1}]$	0.210	5.600	0.021	5.719	0.112	0.307
Concentration	X_0	rS_{Y_0}	rS_{L_0}	C_0		
	29.429 [g/l]	0.000667	0.0172	1.793 [g/l]		
Parameters	μ_m	rK_s	ρ_{co_2}	T		
	0.141 $[\text{h}^{-1}]$	0.00017	0.976 [g/l]	0.05 [h]		
	K_Y	K_L	K_B	Y_{X/co_2}		
	16 [g/l]	0.6 [g/l]	160.32 [g/l]	0.307		

We assume (1) the process state model with 5% model uncertainty; (2) measurements perturbed with 20% measurement errors, that is,

$$Q = \begin{bmatrix} (.05X_0)^2 & 0 & 0 & 0 \\ 0 & (.05rS_{Y_0})^2 & 0 & 0 \\ 0 & 0 & (.05rS_{L_0})^2 & 0 \\ 0 & 0 & 0 & (.05C_0)^2 \end{bmatrix}$$

$$R = \begin{bmatrix} (.2\frac{\mu_m rS_{Y_0}}{rS_{Y_0}+rK_s}X_0)^2 & 0 \\ 0 & (.2C_0)^2 \end{bmatrix}$$

The random state uncertainty w_k and the measurement error v_k are two white-noise processes of intensity Q and R separately as shown in Figure 6.1(a)–(b), the estimation results are shown in Figure 6.1(c)–(f). A good agreement is observed between the true state values and the estimated state values. Therefore based on the developed estimation model in this work that describes cell growth rate as a function of the CO_2 concentration and the base addition rate, the cell concentration, and other state variables can be on-line estimated by applying the EKF from these two available on-line measurement variables, the dissolved CO_2 concentration and the base addition rate.

It is clear that a reasonably good model is essential in order to take full advantage of the extended Kalman filtering theory. At present, however, for real fermentation process the accurate mathematical model, which precisely describe the cell growth and the metabolism of microorganism, is not available due to the high complexity of biological systems as they involve living organisms. Therefore

the values of the model kinetic parameters should be on-line updated in order to match the model to changes in the behavior of the fermentation process, especially the saturation constant K_s in the Monod kinetics which has the big effect on the steady state behavior and dynamic behavior of this process (recall discussions in Section 5.4). Then by incorporating off-line measurements on cell mass and other state variables, an adaptive cell mass estimator can be developed where the model kinetic parameters are updated whenever a new off-line measurement is available, and in between off-line samples these parameters are kept constant and used along with the on-line measurements to estimate the cell mass concentration and other state variables.

Nomenclature

LA	lactic acid	
YEP	mixture of yeast extract and peptone	
B_f	the concentration of feed base solution	[g/l]
$Y_{X/base}$	cell growth yield coefficient on base solution	[g cells/g base]
$K_B = Y_{X/base}B_f$	product of cell yield coefficient on base solution and base feed concentration	[g/l]
C	dissolved carbon dioxide concentration in the medium	[g/l]
Y_{X/co_2}	cell growth yield coefficient on carbon dioxide	[g cells/g co_2]
D_{in1}	yeast extract-peptone influent flow rate	[h^{-1}]
D_{in2}	lactic acid influent flow rate	[h^{-1}]
D_{in3}	base influent flow rate	[h^{-1}]
D_{filter}	filtrate flow rate	[h^{-1}]
D_{out}	product effluent flow rate	[h^{-1}]
D_{co_2}	carbon dioxide offgas evolution rate	[g/l]
K_s	saturation constant in Monod model	[g/l]
S_L	substrate lactic acid concentration	[g/l]
S_{fL}	the feed lactic acid concentration	[g/l]
$Y_{X/L}$	cell growth yield coefficient on substrate lactic acid	[g cells/g LA]

$K_L = Y_{X/L} S_{fL}$	product of cell yield coefficient on lactic acid and lactic acid feed concentration	[g/l]
$rS_L = S_L / S_{fL}$	normalized lactic acid concentration with respect to its feed concentration S_{fL}	
S_Y	substrate YEP concentration	[g/l]
S_{fY}	the feed YEP concentration	[g/l]
$Y_{X/Y}$	cell growth yield coefficient on YEP	[g cells/g YEP]
$K_Y = Y_{X/Y} S_{fY}$	product of cell yield coefficient on YEP and YEP feed concentration	[g/l]
$rS_Y = S_Y / S_{fY}$	normalized YEP concentration with respect to its feed concentration S_{fY}	
$rK_s = K_s / S_{fY}$	normalized saturation constant with respect to YEP feed concentration	
V	the bioreactor volume	[l]
X	cell concentration	[g/l]
$x_k = [X\ rS_Y\ rS_L\ C]^T$	process state vector at time k	
$\hat{x}_{k\|k-1}$	predicted estimate of x_k using measurements up till time $k-1$	
$\hat{x}_k$	filtered estimate of x_k using measurements up till time k	
y_k	measurement vector at time k	
w_k	random model uncertainty	
v_k	measurement error	
L_k	extended Kalman filter gain	
$P_{k\|k-1}$	error covariance matrix of the predicted estimate $\hat{x}_{k\|k-1}$	
P_k	error covariance matrix of the filtered estimate $\hat{x}_k$	

Greek Symbols

μ	specific growth rate	[h^{-1}]
μ_m	maximum specific growth rate	[h^{-1}]
ρ_{co_2}	carbon dioxide gas density	[g/l]

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Chapter 7

High-concentration Cultivation of Propionibacteria in Continuous Bioreactor with Cross-flow Filtration. Part III: Control

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Abstract

This work is based on an industrial application of a continuous bioreactor with cross-flow filtration. The control objective for this biochemical process is to maintain cell concentration at a desired high concentration in order to maximize the production of cell mass. In this work simple frequency-dependent tools such as the relative gain array and the closed loop disturbance gain are used for control structure selection and controllability analysis with respect to disturbance rejection, and the issues on how to select manipulated inputs and how to pair the controlled outputs with the manipulated inputs are discussed. The main consideration and the most interesting work in this paper is the study on the possibility of the partial control of this bioreactor. Based on the controllability analysis with respect to disturbance rejection by using the partial disturbance gain, a 1×1 partial control configuration is proposed as the most efficient and practical way to control this continuous bioreactor.

7.1 Introduction

After studying the modeling and identification of this bioreactor, we now come to the last topic: the control of this bioreactor. In this continuous bioreactor with multiple substrate feeding streams and cross-flow filtration, the main control objective is to keep cell mass X constant at a given high concentration. The growth-limiting substrate yeast extract-peptone concentration rS_Y should be kept small to avoid washout, which is a general requirement for bioreactor operation, and to avoid waste using yeast extract-peptone for economic consideration. The lactic acid concentration rS_L should also be kept low but above its limiting level such that lactic acid does not limit cell growth.

In this paper simple frequency-dependent tools such as the relative gain array, the closed loop disturbance gain, and the partial disturbance gain are used for control structure selection and controllability analysis with respect to disturbance rejection. In Section 7.2, we first study the selection of manipulated inputs (Section 7.2.2), and discuss how to pair the controlled outputs and manipulated inputs for 3×3 decentralized control of this bioreactor (Section 7.2.3). Further in Section 7.3, we study how to develop more efficient and practical control system for this bioreactor, i.e. considering if we may achieve acceptable control performances of all three outputs by controlling only one or two of them. In other words, the possibility of partial control of this continuous bioreactor is studied, which is the main consideration and the most interesting part in this paper.

7.2 Controllability Analysis and Control Structure Selection

In the following we will use a linear plant description of the form

$$y(s) = G(s)u(s) + G_d(s)d(s) \tag{7.1}$$

where G and G_d denote the process and disturbance model, y, u and d are the outputs, manipulated inputs and disturbances, respectively.

The original nonlinear system described by Eqs. (5.9)–(5.11) may be rewritten in vector form:

$$\frac{dy}{dt} = \begin{bmatrix} dX/dt \\ drS_L/dt \\ drS_Y/dt \end{bmatrix} = \begin{bmatrix} (\mu - D_{\text{out}})X \\ -\frac{\mu X}{K_L} - (D_{\text{filter}} + D_{\text{out}})\, rS_L + D_{\text{in2}} \\ -\frac{\mu X}{K_Y} - (D_{\text{filter}} + D_{\text{out}})\, rS_Y + D_{\text{in1}} \end{bmatrix} = f(y,u,d) \tag{7.2}$$

which results in the linearized model

$$y(s) = \underbrace{C(SI - A)^{-1}B}_{G}\, u(s) + \underbrace{C(SI - A)^{-1}B_d}_{G_d}\, d(s) \tag{7.3}$$

where

$$A = \left.\frac{\partial f}{\partial y}\right|_{\bar{y},\bar{u},\bar{d}}, \quad B = \left.\frac{\partial f}{\partial u}\right|_{\bar{y},\bar{u},\bar{d}}, \quad B_d = \left.\frac{\partial f}{\partial d}\right|_{\bar{y},\bar{u},\bar{d}}, \quad C = I_{3\times 3}$$

Here the subscript $\bar{y}, \bar{u}, \bar{d}$ means that matrices A, B, and B_d are calculated by the substitution of steady-state values for y, u and d.

Because most controllability measures (except for the relative gain array (RGA)) are scaling dependent, it is crucial that the variables are scaled properly before we do the controllability analysis. In general, the variables are scaled to be within the interval -1 to 1, i.e. their allowed or expected magnitudes are normalized to be less than 1 at each frequency. To achieve this we scale directly the transfer matrices G and G_d with respect to the maximum allowed or expected ranges of variables y, u and d under the assumption that the allowed or expected magnitudes of these signals y, u and d do not vary with frequency.

For the studied continuous bioreactor,
the allowed maximum output changes are

$$\overline{\Delta X} = 10\%\,\overline{X}; \quad \overline{\Delta rS_L} = 30\%\,\overline{rS_L}; \quad \overline{\Delta rS_Y} = 20\%\,\overline{rS_Y}$$

the allowed maximum input changes are

$$\overline{\Delta D_{\mathrm{in1}}} = 50\%\,\overline{D_{\mathrm{in1}}}; \quad \overline{\Delta D_{\mathrm{in2}}} = 50\%\,\overline{D_{\mathrm{in2}}}; \quad \overline{\Delta D_{\mathrm{in3}}} = 50\%\,\overline{D_{\mathrm{in3}}}$$

$$\overline{\Delta D_{\mathrm{filter}}} = 50\%\,\overline{D_{\mathrm{filter}}}; \quad \overline{\Delta D_{\mathrm{out}}} = 50\%\,\overline{D_{\mathrm{out}}}$$

the expected maximum disturbance changes are

$$\overline{\Delta D_{\mathrm{in1_d}}} = 5\%\,\overline{D_{\mathrm{in1}}}; \quad \overline{\Delta D_{\mathrm{in2_d}}} = 5\%\,\overline{D_{\mathrm{in2}}}; \quad \overline{\Delta D_{\mathrm{in3_d}}} = 5\%\,\overline{D_{\mathrm{in3}}}$$

$$\overline{\Delta D_{\mathrm{filter_d}}} = 5\%\,\overline{D_{\mathrm{filter}}}; \quad \overline{\Delta D_{\mathrm{out_d}}} = 5\%\,\overline{D_{\mathrm{out}}}$$

$$\overline{\Delta K_Y} = 10\%\,\overline{K_Y}; \quad \overline{\Delta rK_s} = 10\%\,\overline{rK_s}; \quad \overline{\Delta \mu_m} = 10\%\,\overline{\mu_m}$$

Here, in addition to possible disturbances in the manipulated inputs, we have included disturbances in the model parameters K_Y, rK_s and μ_m which may stem

from variations in the environment conditions such as temperature, pH and aeration rate etc. In the following the overline ($^-$) used to denote steady-state values will be omitted to simplify notation.

The desired steady state values and parameters, which will be used for both frequency response and time response simulations, are listed in Table 7.1.

Table 7.1: Steady State Data

Dilution rate	D_{in1}	D_{in2}	D_{in3}	D_{filter}	D_{out}
$[\text{h}^{-1}]$	0.210	5.600	0.021	5.719	0.112
Concentration	X	rS_L	rS_Y		
	29.429 [g/l]	0.0172	0.000667		
Parameters	μ_m	rK_s	K_L	K_Y	
	0.141 $[\text{h}^{-1}]$	0.00017	0.6 [g/l]	16 [g/l]	

In general, this continuous bioreactor has no poles or transmission zeros in the right half plane (RHP). Therefore the bioreactor is stable at all nontrivial steady states and there are no fundamental problems related with instability, inverse responses or inherent bandwidth limitations.

7.2.1 Measures for evaluating controllability

The most widespread controllability measure is probably the relative gain array (RGA) which was introduced by Bristol (1966). In this chapter, we initially focus on the use of the RGA as a measure of the interactions caused by decentralized diagonal control, and as a tool for control structure selection. The properties of the RGA are well known (e.g. Grosdidier et al., 1985). The most important for our purpose is that the RGA depends on the plant model only and is independent of scaling in inputs or outputs. For $n \times n$ plant $G(s)$ the RGA matrix can be computed using the formula,

$$\text{RGA}(s) = G(s) \times [G^{-1}(s)]^T \tag{7.4}$$

where the $\times$ symbol denotes element by element multiplication (Hadamard product).

To evaluate the disturbance sensitivity, we consider the closed loop disturbance gain (CLDG) proposed by Hovd and Skogestad (1992), which is the appropriate measure when we use decentralized control. For a disturbance d_k and

an output y_i, the CLDG is defined by

$$\text{CLDG}_{ik}(s) = g_{ii}(s)[G(s)^{-1}G_d(s)]_{ik} \tag{7.5}$$

A matrix of CLDG's may be computed from

$$\text{CLDG}(s) = G_{diag}(s)G^{-1}(s)G_d(s) \tag{7.6}$$

where G_{diag} consists of the diagonal elements of G. The CLDG is scaling dependent, as it depends on the expected magnitude of disturbances and outputs. Actually, this is reasonable since the CLDG is a performance measure, which generally is scaling dependent.

Summary of controllability rules.

The following rules are useful for pairing inputs and outputs:

Rule 1. Avoid plants (designs) with large RGA-values (in particular at frequencies near cross-over). This rule applies for any controller, not only to decentralized control (Skogestad and Morari, 1987).

Rule 2. To avoid instability caused by interactions at low frequencies one should avoid pairings with negative steady-state RGA elements (e.g. Skogestad and Postlethwaite, 1996, p.435).

Rule 3. Avoid pairings with "significant" RHP-zeros (RHP-zeros close to the origin) in $g_{ij}(s)$ because otherwise this loop may go unstable if left by itself (with the other loops open) when decentralized controllers are used.

7.2.2 Selection of manipulated inputs

In this continuous bioreactor the main control objective is to control cell mass X constant at a desired high concentration. We have three output variables X, rS_L and rS_Y; the most important controlled output of them is X. We have five manipulated inputs as shown in Figure 7.1. Since the pH is already controlled by the base addition rate D_{in3}, and the reactor volume is assumed constant such that $D_{\text{in1}} + D_{\text{in2}} + D_{\text{in3}} = D_{\text{filter}} + D_{\text{out}}$, three independent manipulated variables (D_{in1}, D_{in2} and D_{out} or D_{in1}, D_{filter} and D_{out}) remain. Generally large flow rates are used to control reactor volume, then we have two alternative manipulated inputs D_{in2} and D_{filter} for reactor volume control and therefore two alternative control strategies exist for controlling this bioreactor:

Alternative control strategy (a):

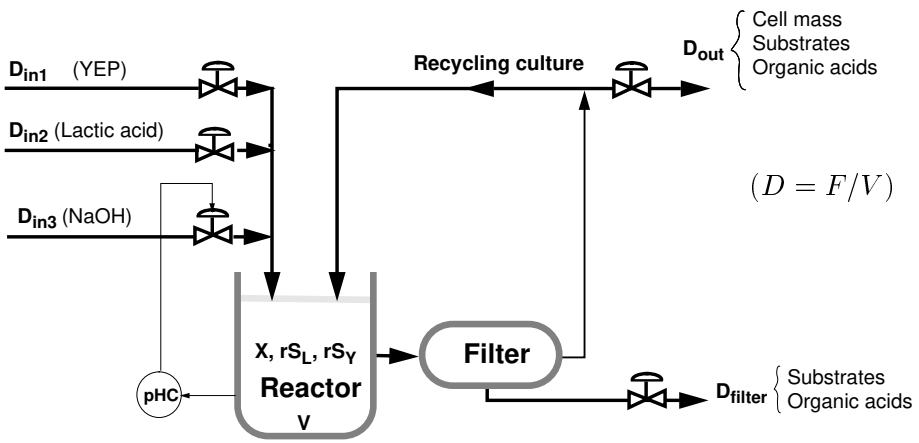


Figure 7.1: A continuous bioreactor with cross-flow filtration

$$y = \begin{bmatrix} X \\ rS_L \\ rS_Y \end{bmatrix}; \quad u = \begin{bmatrix} D_{\text{in1}} \\ \mathbf{D}_{\text{in2}} \\ D_{\text{out}} \end{bmatrix}; \quad d = \begin{bmatrix} D_{\text{in1}_\text{d}} \\ D_{\text{in2}_\text{d}} \\ D_{\text{out}_\text{d}} \\ K_Y \\ rK_s \\ \mu_m \end{bmatrix} \tag{7.7}$$

Alternative control strategy (b):

$$y = \begin{bmatrix} X \\ rS_L \\ rS_Y \end{bmatrix}; \quad u = \begin{bmatrix} D_{\text{in1}} \\ \mathbf{D}_{\text{filter}} \\ D_{\text{out}} \end{bmatrix}; \quad d = \begin{bmatrix} D_{\text{in1}_\text{d}} \\ D_{\text{filter}_\text{d}} \\ D_{\text{out}_\text{d}} \\ K_Y \\ rK_s \\ \mu_m \end{bmatrix} \tag{7.8}$$

The steady-state gain matrices in terms of scaled variables for the two alternative control strategies are:

$$G_a(0) = \begin{bmatrix} 5.09 & -0.09 & -5.46 \\ -93.15 & 93.15 & 8.41 \\ 0.00 & 0.00 & 12.21 \end{bmatrix} \tag{7.9}$$

$$G_b(0) = \begin{bmatrix} 5.09 & -0.09 & -5.46 \\ -96.64 & 95.12 & 10.28 \\ 0.00 & 0.00 & 12.21 \end{bmatrix} \tag{7.10}$$

$$(G_d)_a(0) = \begin{bmatrix} 0.51 & -0.01 & -0.55 & 1.00 & -0.02 & 0.09 \\ -9.31 & 9.31 & 0.84 & -18.29 & 0.34 & -1.68 \\ 0.00 & 0.00 & 1.22 & 0.00 & 0.50 & -2.44 \end{bmatrix} \tag{7.11}$$

$$(G_d)_b(0) = \begin{bmatrix} 0.51 & -0.01 & -0.55 & 1.00 & -0.02 & 0.09 \\ -9.66 & 9.51 & 1.03 & -18.29 & 0.34 & -1.68 \\ 0.00 & 0.00 & 1.22 & 0.00 & 0.50 & -2.44 \end{bmatrix} \tag{7.12}$$

We note immediately that the steady state gains from the manipulated inputs to output X in G_a are almost same as in G_b, and the steady state gains from the manipulated inputs to output rS_Y are also unchanged from G_a to G_b. The steady state gains from the manipulated inputs to output rS_L in control strategy (b) are a little bit higher than in control strategy (a).

Now, we look at the steady-state RGA values and CLDG values,

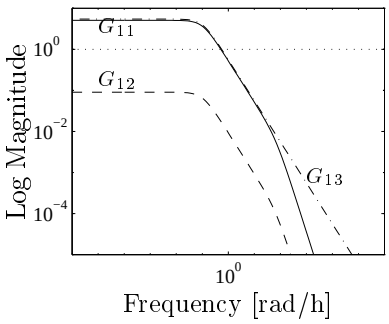
$$\mathrm{RGA}_a(0) = \begin{bmatrix} 1.02 & -0.02 & 0.00 \\ -0.02 & 1.02 & 0.00 \\ 0.00 & 0.00 & 1.00 \end{bmatrix} \tag{7.13}$$

$$\mathrm{RGA}_b(0) = \begin{bmatrix} 1.02 & -0.02 & 0.00 \\ -0.02 & 1.02 & 0.00 \\ 0.00 & 0.00 & 1.00 \end{bmatrix} \tag{7.14}$$

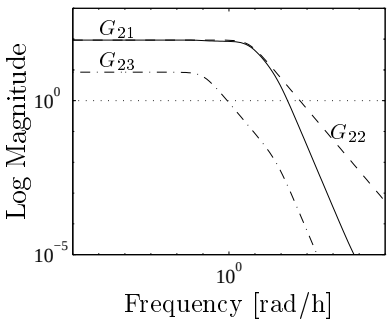
$$\mathrm{CLDG}_a(0) = \begin{bmatrix} 0.51 & 0.00 & 0.00 & 1.00 & 0.21 & -1.02 \\ 0.00 & 9.31 & 0.00 & 0.01 & 3.81 & -18.63 \\ 0.00 & 0.00 & 1.22 & 0.00 & 0.50 & -2.44 \end{bmatrix} \tag{7.15}$$

$$\mathrm{CLDG}_b(0) = \begin{bmatrix} 0.51 & 0.00 & 0.00 & 1.00 & 0.21 & -1.02 \\ 0.00 & 9.51 & 0.00 & 0.70 & 3.88 & -18.95 \\ 0.00 & 0.00 & 1.22 & 0.00 & 0.50 & -2.44 \end{bmatrix} \tag{7.16}$$

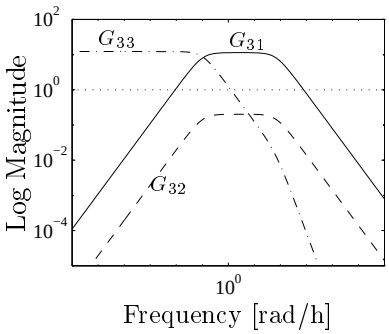
We see that the steady state RGA values are the same in the two alternative control strategies, and very small differences exist in the steady state CLDG between these alternative control strategies. By looking at the frequency-dependent plots of G, G_d, RGA and CLDG (only G, G_d, RGA and CLDG of alternative control strategy (a) are shown in Figures 7.2 and 7.3), the frequency responses of these two alternative control strategies are quite similar to each other. From the CLDG we also see that, in regarding to the desired steady state as given in Table 7.1,



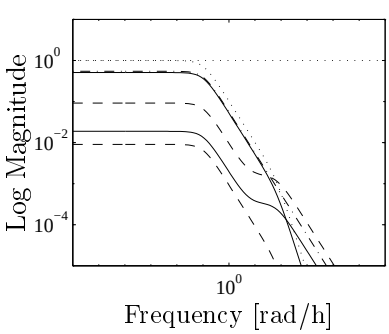
(a) G_{11}–G_{13}



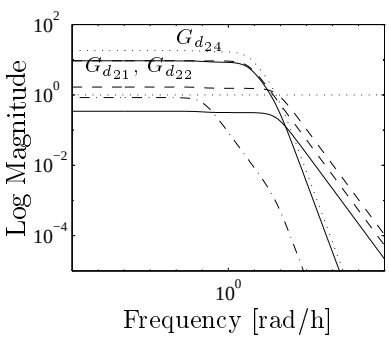
(b) G_{21}–G_{23}



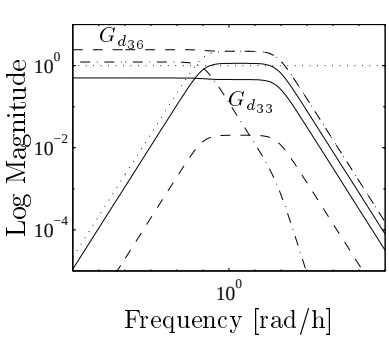
(c) G_{31}–G_{33}



(d) $G_{d_{11}}$–$G_{d_{16}}$



(e) $G_{d_{21}}$–$G_{d_{26}}$



(f) $G_{d_{31}}$–$G_{d_{36}}$

Figure 7.2: Frequency -dependent plots of G and G_d for control strategy (a)

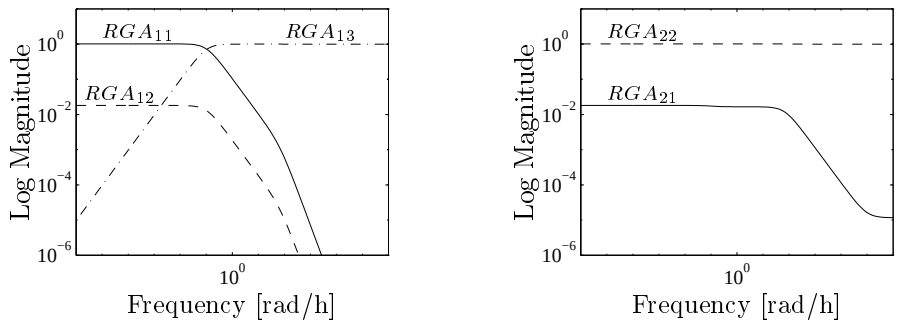
no problems with input constraints exist in this plant as all $|g_{ii}| > |\text{CLDG}_{ik}|$ for $i = 1, 2, 3$ and $k = 1, ..., 6$ (as shown in Figure 7.3(d)–(f)). In addition, the CLDG shows that for all three outputs, the most difficult disturbance to reject is changes in the parameter μ_m. The required bandwidths for controlling X, rS_L and rS_Y respectively are also same in the two alternative control strategies.

Therefore **we conclude** that, based on the controllability analysis, the substrate lactic acid feed flow rate D_{in2} and the filtrate bleeding rate D_{filter} have the same effect on the outputs X, rS_L and rS_Y from a control point of view. On the other hand, from a biotechnology point of view, for a given microorganism the ratio $D_{\text{in1}}/D_{\text{in2}}$ should be nearly constant in order to get the balanced medium for cell growth, and thus D_{in2} should not be used for volume control to avoid unnecessary change in substrates. Consequently we choose alternative control strategy (a): D_{filter} is used to control the reactor volume and the remaining three flow rates, D_{in1}, D_{in2} and D_{out}, control the outputs X, rS_L and rS_Y.

7.2.3 Pairing of controlled and manipulated variables

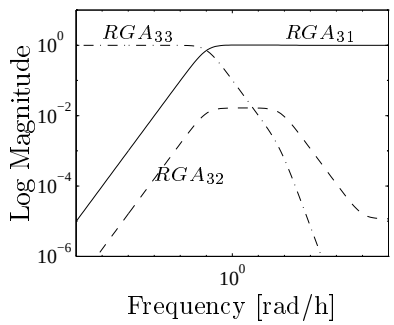
We now proceed with the control structure selection by using the pairing rules described in Section 7.2.1. The steady-state gains from D_{in1} to rS_Y and from D_{in2} to rS_Y are zeros which means that at steady state rS_Y only depends on D_{out}. The cell concentration X is sensitive to both the feed limiting substrate flow rate D_{in1} and the cell production flow rate D_{out}, but rather insensitive to D_{in2}. Flow rates D_{in1} and D_{in2} have more larger effect on rS_L. From the steady state RGA values, we first see that no significant interaction problems exist between these control loops of X, rS_L and rS_Y. The RGA values of this plant are small, thus Rule 1 is satisfied. Based on Rule 2, we should avoid *pairing ij* with negative values of the steady-state RGA, and thus using D_{in1} to control rS_L should be avoided even though it has a large steady-state gain. From Rule 3, we should avoid the pairings $D_{\text{in1}} - rS_Y$ and $D_{\text{in2}} - rS_Y$ since their corresponding steady-state gains g_{31} and g_{32} have a zero at the origin, respectively.

In summary, we choose D_{in1} to control X, D_{in2} to control rS_L and D_{out} to control rS_Y, in order to have positive steady-state RGA values. Furthermore, this plant (corresponding to the given pairings $D_{\text{in1}} - X$, $D_{\text{in2}} - rS_L$ and $D_{\text{out}} - rS_Y$) is Decentralized Integral Controllable (DIC) since $\sqrt{\text{RGA}_{11}(0)} + \sqrt{\text{RGA}_{22}(0)} + \sqrt{\text{RGA}_{33}(0)} > 1$ (Yu and Fan, 1990). In addition, we have earlier studied the effects of the parametric uncertainty in the Monod parameter rK_s and changes

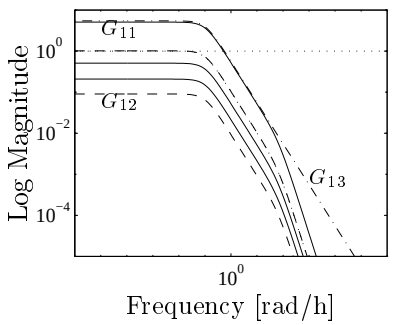


(a) RGA_{11}–RGA_{13}

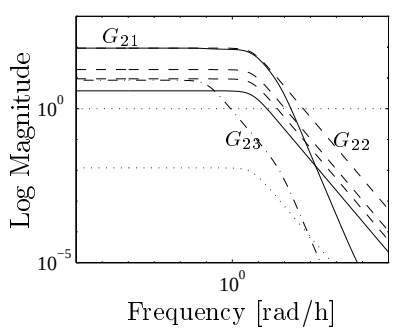
(b) RGA_{21}–RGA_{23}



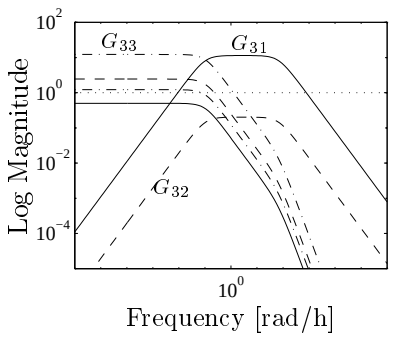
(c) RGA_{31}–RGA_{33}



(d) $CLDG_{11}$–$CLDG_{16}$



(e) $CLDG_{21}$–$CLDG_{26}$



(f) $CLDG_{31}$–$CLDG_{36}$

Figure 7.3: Frequency-dependent plots of RGA and CLDG for control strategy (a)

in the operating point on the choice of control structure, and we found that the *choice of parings* appears to be unaffected either by changes in the operating point or by the parametric uncertainty in rK_s (Zhao and Skogestad, 1994).

One may argue that it seems that there is an alternative control structure with pairings $D_{\text{in1}} - rS_Y$, $D_{\text{in2}} - rS_L$ and $D_{\text{out}} - X$ since these pairings corresponding to RGA elements close to 1 in the higher frequency region (see Figure 7.3(a)–(c)). However, the obvious reason for not choosing the pairing $D_{\text{in1}} - rS_Y$ is that at steady state rS_Y is independent of D_{in1} as above discussed. Using D_{out} to control X is unfavorable for maximization of the cell productivity ($P = D_{\text{out}}X$) as X varies in the opposite direction to D_{out}, i.e. to increase X we have to decrease D_{out} (recall Section 5.4.2 and Figure 5.5(a) for the steady state relationship between X and D_{out}).

We have so far discussed the control structure selection for 3×3 decentralized control of this bioreactor. However, a practical implementation of a 3×3 control system of a bioreactor is expensive and difficult; mainly due to the lack of reliable on-line sensors which can measure all three outputs, cell concentration and substrate concentrations. On the other hand, it may not be necessary to control all three outputs, as the main objective of operating this bioreactor is to continuously get constant high concentration of cells. Therefore the main consideration in this paper is on whether one may achieve acceptable control performances of all three outputs by controlling only one or two of them, i.e. the plant is partially controlled.

7.3 Partial Control System

7.3.1 Measures for evaluating controllability

As discussed above, we are actually faced with a "partial control problem", that is to use a subset of the available inputs to control a subset of the outputs. We now close only one loop (1×1 control) or two loops (2×2 control) and define $y = [y_1 \quad y_2]^T$ and $u = [u_1 \quad u_2]^T$, where

y_1— uncontrolled outputs

y_2— controlled outputs

u_1— unused inputs

u_2— manipulated inputs used to control y_2

Therefore the model $y = Gu + G_d d$ is rearranged and partitioned into two parts,

$$y_1 = G_{11}u_1 + G_{12}u_2 + G_{d1}d \tag{7.17}$$

$$y_2 = G_{21}u_1 + G_{22}u_2 + G_{d2}d \tag{7.18}$$

The problem is to select the controlled outputs (y_2), and the corresponding manipulated inputs (u_2) such that :

1. Use of u_2 to control y_2 should yield satisfactory control performance.
2. With these control loops closed the uncontrolled outputs y_1 should be relatively insensitive to disturbances d.

To evaluate both these issues a controllability analysis of the alternative structures needs to be performed. A useful tool when considering issue 2 is the partial disturbance gain which is the effect of a disturbance on uncontrolled outputs when the plant is under partial control. In Chapter 3, we have defined the partial disturbance gain in terms of the induced 2-norm and we have also discussed the characteristics of the partial disturbance gain. For more details, please refer to Section 3.2.

For a square plant with only one output y_2 perfectly controlled (1×1 control), the sensitivity of the uncontrolled outputs y_1 to a disturbance d_k is given by Eq. (3.30) in Appendix of Chapter 3:

$$P_{d1} = G_{d1} - G_{12}G_{22}^{-1}G_{d2} \tag{7.19}$$

Next, consider the case with two outputs (y_2 is a vector) perfectly controlled (2×2 control). This corresponds to having one input u_1 "unused" and the sensitivity of the uncontrolled output y_1 to a disturbance d_k is given by Eq. (3.36) in Appendix of Chapter 3:

$$P_{d1} = \frac{[G^{-1}G_d]_1}{[G^{-1}]_{11}} \tag{7.20}$$

For simultaneous disturbances d_k, $k = 1, 2, ...$, the worst overall effect may be evaluated by the largest singular value of partial disturbance gain matrix, $\bar{\sigma}(P_d)$ which is equal to $\|P_{d1}\|_{i2}$. Skogestad and Wolff (1992) proposed using the "combined partial disturbance gain (CPDG)", i.e. by taking the sum of the partial disturbance gain element magnitudes for each "pairing" to evaluate the worst overall effect of simultaneous disturbances on uncontrolled outputs,

$$\text{CPDG}_1 = \sum_k |[P_{d1}]_{ik}| \tag{7.21}$$

The objective of the controllability analysis in this section is to find the best partial control configuration so that we can get the acceptable disturbance sensitivity for the uncontrolled outputs even under simultaneous disturbances. To attain this, we prefer such pairing corresponding to the smallest $\|P_{d1}\|_{i2}$.

7.3.2 1×1 partial control

For this 3×3 plant, there are nine alternative 1×1 partial control configurations. The overall effect of simultaneous disturbances on uncontrolled outputs y_1 are given by $\|P_{d1}\|_{i2}$, both with the steady-state values listed in Table 7.2 and as the function of frequency shown in Figure 7.4. The steady-state CPDG values are also given in Table 7.2.

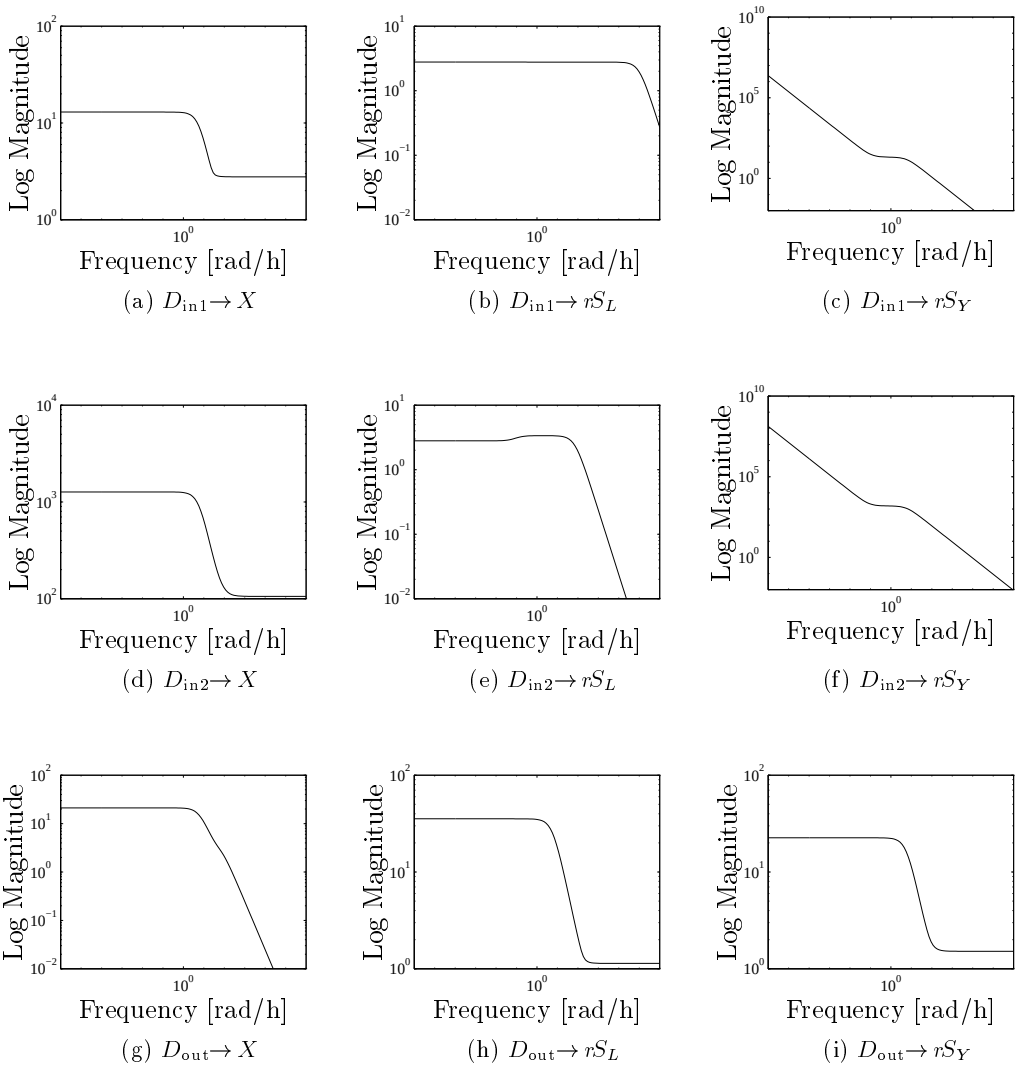
Table 7.2: Partial disturbance gain (1×1 control)

Controlled pairing			CPDG$_1$			$\|P_{d1}\|_{i2}$
No.	u_2	y_2	$\Sigma_k \frac{\partial X}{\partial d_k}$	$\Sigma_k \frac{\partial rS_L}{\partial d_k}$	$\Sigma_k \frac{\partial rS_Y}{\partial d_k}$	
1	$D_{\text{in}1}$	X		18.31	4.16	12.97
2	$D_{\text{in}1}$	rS_L	1.00		4.16	2.78
3	$D_{\text{in}1}$	rS_Y	∞	∞		∞
4	$D_{\text{in}2}$	X		2200	4.16	1270
5	$D_{\text{in}2}$	rS_L	2.14		4.16	2.80
6	$D_{\text{in}2}$	rS_Y	∞	∞		∞
7	D_{out}	X		36.43	6.09	21.13
8	D_{out}	rS_L	23.64		53.57	35.43
9	D_{out}	rS_Y	2.72	36.91		22.56

From the steady state values and frequency responses, the best one among these nine different partial control configurations is the $D_{\text{in}1} \rightarrow rS_L$ – configuration (No. 2), which is corresponding to the smallest $\|P_{d1}\|_{i2}$.

Configurations No. 3 and 6 with rS_Y controlled by $D_{\text{in}1}$ or $D_{\text{in}2}$ are not feasible as we can see that the disturbance sensitivities of uncontrolled outputs X and rS_L are infinite, because rS_Y only depends on D_{out}.

Configurations No. 1, No. 4 and No. 7–9 are unacceptable with large sensitivities of uncontrolled outputs to disturbances. In particular, the configuration No. 4 with X controlled by $D_{\text{in}2}$ should be avoided to use because X is rather



(a) $D_{\text{in}1} \rightarrow X$ (b) $D_{\text{in}1} \rightarrow rS_L$ (c) $D_{\text{in}1} \rightarrow rS_Y$

(d) $D_{\text{in}2} \rightarrow X$ (e) $D_{\text{in}2} \rightarrow rS_L$ (f) $D_{\text{in}2} \rightarrow rS_Y$

(g) $D_{\text{out}} \rightarrow X$ (h) $D_{\text{out}} \rightarrow rS_L$ (i) $D_{\text{out}} \rightarrow rS_Y$

Figure 7.4: Frequency-dependent plots of $\|P_{d1}\|_{i2}$ for 1×1 partial control

insensitive to changes in $D_{\text{in}2}$ (the steady-state gain from $D_{\text{in}2}$ to X (G_{12}) is only -0.09 which is about 60 times smaller than G_{11} or G_{13} as given in Eq. (7.9), see also Figure 7.2(a) for the corresponding frequency responses).

Configurations No. 2 and 5 are similar in terms of their values of $\|P_{d1}\|_{i2}$, but the configurations No. 2 is better in terms of the effect of the disturbances on the cell concentration X which is the most important output variable. For the configuration No. 2 where $D_{\text{in}1}$ is used to control rS_L, the overall effect of simultaneous disturbances on X, although it is uncontrolled, is only 1.00 in terms of CPDG which means that even under the worst case (all disturbances are in maximum intensities), the cell concentration can still be kept within its allowed change range. The reason for the good performance achieved with the configuration No. 2 is that $D_{\text{in}1}$ has larger effects on both X and rS_L (see $G(0)$ in Eq. (7.9) and frequency plots of G in Figure 7.2). From the biotechnological point of view, this is also reasonable since the control of carbon source concentration rS_L is extremely important in achieving the process objective (e.g. O'Connor et al., 1991). The practical implementation of configuration No. 2 is also possible since on-line measurement of lactic acid concentration is easier than cell mass or yeast extract-peptone concentration.

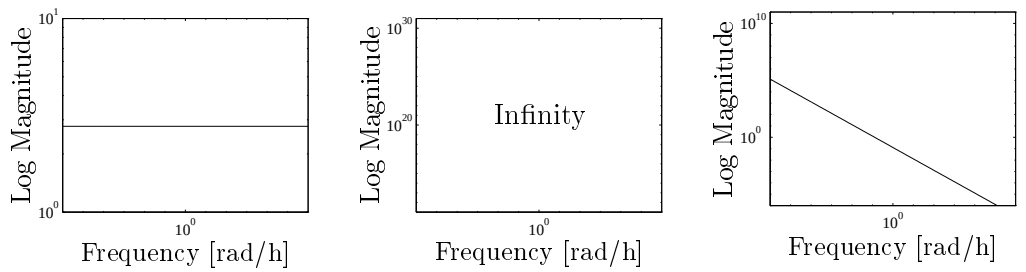
7.3.3 2×2 partial control

For this 3×3 plant, the use of 2×2 partial control means that only one output is uncontrolled. There exist nine different 2×2 control configurations. Table 7.3 shows $\|P_{d1}\|_{i2}$ and CPDG values at steady state. The frequency-dependent plots of $\|P_{d1}\|_{i2}$ for the nine configurations are shown in Figure 7.5. The best 2×2 control system is No. 6 with $u_2 = [D_{\text{in}1}\, D_{\text{out}}]^T$ and $y_2 = [rS_L\, rS_Y]^T$. It gives almost acceptable sensitivity of the uncontrolled X to disturbances.

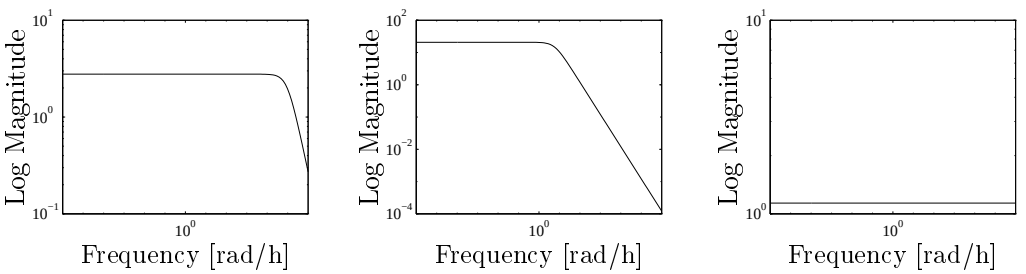
However, comparing with the case by 1×1 control, we see that cell concentration X is more sensitive to disturbances than in the configuration No. 2 in Table 7.2. In other words, the disturbance sensitivity of the uncontrolled output cell concentration X becomes worse as we switch from 1×1 control to 2×2 control.

Table 7.3: Partial disturbance gain (2×2 control)

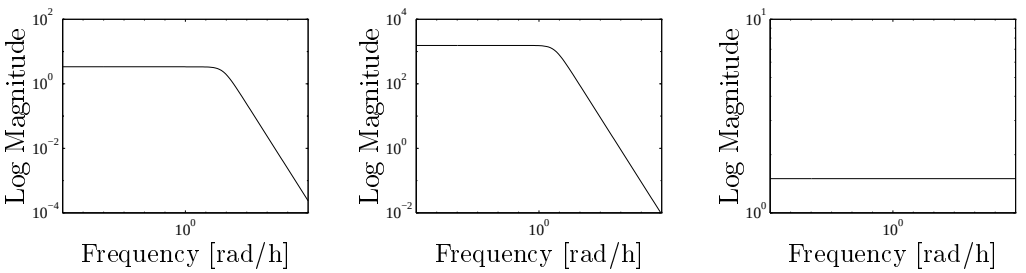
Controlled pairing			CPDG$_1$			$\|P_{d1}\|_{i2}$
No.	u_2	y_2	$\Sigma_k \frac{\partial X}{\partial d_k}$	$\Sigma_k \frac{\partial rS_L}{\partial d_k}$	$\Sigma_k \frac{\partial rS_Y}{\partial d_k}$	
1.	$\begin{bmatrix} D_{\text{in1}} \\ D_{\text{in2}} \end{bmatrix}$	$\begin{bmatrix} X \\ rS_L \end{bmatrix}$			4.16	2.78
2.	$\begin{bmatrix} D_{\text{in1}} \\ D_{\text{in2}} \end{bmatrix}$	$\begin{bmatrix} X \\ rS_Y \end{bmatrix}$		∞		∞
3.	$\begin{bmatrix} D_{\text{in1}} \\ D_{\text{in2}} \end{bmatrix}$	$\begin{bmatrix} rS_L \\ rS_Y \end{bmatrix}$	∞			∞
4.	$\begin{bmatrix} D_{\text{in1}} \\ D_{\text{out}} \end{bmatrix}$	$\begin{bmatrix} X \\ rS_L \end{bmatrix}$			4.17	2.78
5.	$\begin{bmatrix} D_{\text{in1}} \\ D_{\text{out}} \end{bmatrix}$	$\begin{bmatrix} X \\ rS_Y \end{bmatrix}$		31.2		20.80
6.	$\begin{bmatrix} D_{\text{in1}} \\ D_{\text{out}} \end{bmatrix}$	$\begin{bmatrix} rS_L \\ rS_Y \end{bmatrix}$	1.71			1.14
7.	$\begin{bmatrix} D_{\text{in2}} \\ D_{\text{out}} \end{bmatrix}$	$\begin{bmatrix} X \\ rS_L \end{bmatrix}$			6.02	3.37
8.	$\begin{bmatrix} D_{\text{in2}} \\ D_{\text{out}} \end{bmatrix}$	$\begin{bmatrix} X \\ rS_Y \end{bmatrix}$		2767		1547
9.	$\begin{bmatrix} D_{\text{in2}} \\ D_{\text{out}} \end{bmatrix}$	$\begin{bmatrix} rS_L \\ rS_Y \end{bmatrix}$	2.69			1.50



(a) Configuration No. 1 (b) Configuration No. 2 (c) Configuration No. 3



(d) Configuration No. 4 (e) Configuration No. 5 (f) Configuration No. 6



(g) Configuration No. 7 (h) Configuration No. 8 (i) Configuration No. 9

Figure 7.5: Frequency responses of $\|P_{d1}\|_{i2}$ for 2×2 partial control. Configuration No. 1–No. 9 are specified in Table 7.3.

7.4 Time Response Simulation Results

In this section we present nonlinear simulation results to compare with the controllability analysis results presented in the previous sections. In all simulations simple PI controllers are used for the controlled outputs and we consider the step responses to the combined disturbances in parameters K_Y, rK_s and μ_m as shown in Fig. 7.6(d).

From simulation results we find:

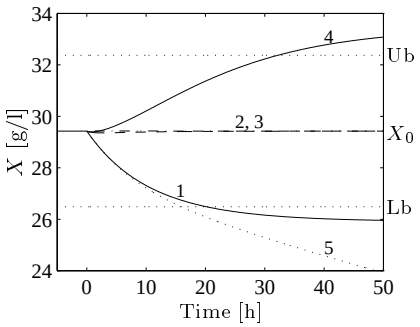
The system cannot be left uncontrolled, otherwise, as shown in Fig. 7.6 (curves 1), after a long time (for example during the weekend in a practical situation) the cell concentration X may decrease below its lower bound (Lb); and the substrate concentration, especially rS_L, will be much high with loss of substrate.

As shown in Fig. 7.6 (curves 2), if all outputs X, rS_L and rS_Y were on-line measurable, then the 3×3 decentralized control system (X is controlled by D_{in1}, rS_L is controlled by D_{in2} and rS_Y is controlled by D_{out}) could work well against the combined disturbances in parameters as all three outputs remain at their desired steady states without violating the input constraints.

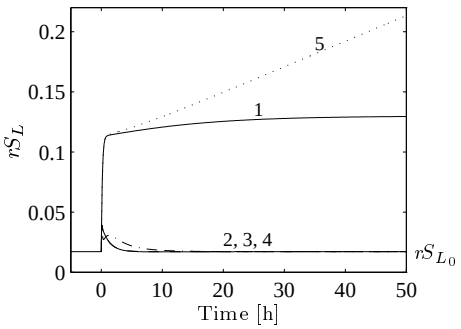
With the 1×1 partial control configuration $D_{\text{in1}} \rightarrow rS_L$ (curves 3 in Figure 7.6), rS_L is perfectly controlled without violating the input constraint of D_{in1}; the uncontrolled X almost goes back to its desired value after several hours so that the main operation objective of this fermentation process has been achieved. The deviation of uncontrolled rS_Y from its desired value is large but tolerable.

With the 2×2 partial control configuration $u_2 = [D_{\text{in1}}\, D_{\text{out}}]^T$ and $y_2 = [rS_L\, rS_Y]^T$ (curves 4 in Figure 7.6), we find no improvement compared to the 1×1 configuration $D_{\text{in1}} \rightarrow rS_L$ except the rS_L returns quicker to its desired value, and after long transition process rS_Y may be controlled at its desired value. However the uncontrolled X exceeds its upper bound (Ub).

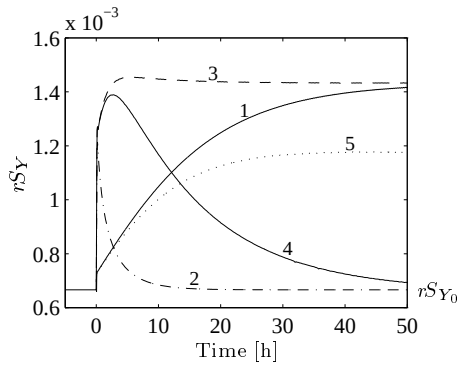
The partial control configuration D_{in1} (or D_{in2})$\rightarrow rS_Y$ with D_{out} left in manual should be avoided, otherwise as shown in Fig 7.6 (curves 5) the uncontrolled X decreases quickly and eventually down to zero and then the reaction will stop: D_{in1} has to be continuously lowered in order to try to keep rS_Y constant, however, it is not possible to keep rS_Y constant in the long run while D_{out} is unchanged because rS_Y is independent of D_{in1} at steady state, and finally D_{in1} will reach zero and results in a failure of the reactor.



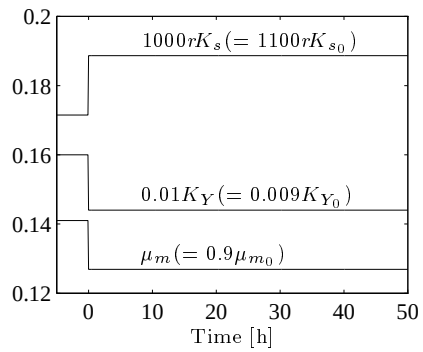
(a) Output X



(b) Output rS_L



(c) Output rS_Y



(d) Combined disturbances

line type	configuration type
curves 1	uncontrolled
curves 2	3×3 control
curves 3	1×1 control $D_{\text{in1}} \rightarrow rS_L$
curves 4	2×2 control
curves 5	1×1 control $D_{\text{in1}} \rightarrow rS_Y$

Figure 7.6: Time responses for the combined step disturbances in parameters K_Y, rK_s and μ_m

7.5 Conclusions

Based on both linear controllability analysis and simulation results, we concluded: (1) The 3×3 decentralized control system, where X is controlled by D_{in1}, rS_L is controlled by D_{in2} and rS_Y is controlled by D_{out}, could work well to maintain all three outputs at their desired steady states against the combined disturbances in parameters and without violating the input constraints, but it does not seem to be realistic due to the lack of reliable on-line sensors which can measure all three outputs.
(2) The 2×2 partial control configuration with $u_2 = [D_{\text{in1}}\, D_{\text{out}}]^T$ and $y_2 = [rS_L\, rS_Y]^T$, although it is the best among the nine alternative 2×2 partial control configurations for this plant, does not improve the output performance compared to that achieved by the 1×1 configuration $D_{\text{in1}} \rightarrow rS_L$. This is because of the interactions between these two control loops.
(3) We suggest to control this continuous bioreactor with cross-flow filtration by using a 1×1 partial control configuration ($D_{\text{in1}} \rightarrow rS_L$) where the yeast extract-peptone feed flow rate D_{in1} is used to control lactic acid concentration rS_L. The reason is that (a) the main operation objective is achieved as X is kept at its desired value against the combined disturbances in parameters; and (b) the practical implementation is possible as on-line measurement of lactic acid concentration is easier than cell mass and yeast extract-peptone concentration.

A comparison shows that the time-response simulation results are consistent with the linear controllability analysis. Therefore, as found earlier in Chapter 3, this industrial application example shows that the partial disturbance gain is an effective tool for controllability analysis which is independent of the controller design and avoids time consuming on detailed simulations.

Nomenclature

LA	lactic acid	
YEP	mixture of yeast extract and peptone	
D_{in1}	yeast extract-peptone influent flow rate	$[\text{h}^{-1}]$
D_{in2}	lactic acid influent flow rate	$[\text{h}^{-1}]$
D_{in3}	base influent flow rate	$[\text{h}^{-1}]$
D_{filter}	filtrate flow rate	$[\text{h}^{-1}]$
D_{out}	product effluent flow rate	$[\text{h}^{-1}]$

K_s	saturation constant in Monod model	[g/l]
S_L	substrate LA concentration	[g/l]
S_{fL}	the feed LA concentration	[g/l]
$Y_{X/L}$	cell growth yield coefficient on LA	[g cells/ g LA]
$K_L = Y_{X/L} S_{fL}$	product of cell yield coefficient on LA and LA feed concentration	[g/l]
$rS_L = S_L / S_{fL}$	normalized LA concentration with respect to its feed concentration S_{fL}	
S_Y	substrate YEP concentration	[g/l]
S_{fY}	the feed YEP concentration	[g/l]
$Y_{X/Y}$	cell growth yield coefficient on YEP	[g cells/ g YEP]
$K_Y = Y_{X/Y} S_{fY}$	product of cell yield coefficient on YEP and YEP feed concentration	[g/l]
$rS_Y = S_Y / S_{fY}$	normalized YEP concentration with respect to its feed concentration S_{fY}	
$rK_s = K_s / S_{fY}$	normalized saturation constant with respect to YEP feed concentration	
V	the bioreactor volume	[l]
X	cell concentration	[g/l]

d	disturbance
G	plant transfer function matrix
G_d	disturbance transfer function matrix
P_d	partial disturbance gain
$\|P_d\|_{i2}$	induced 2-norm of P_d
u	plant input
y	plant output

Greek Symbols

$\bar{\sigma}(P_d)$	largest singular value of P_d $(= \|P_d\|_{i2})$	
μ	specific growth rate	[h^{-1}]
μ_m	maximum specific growth rate	[h^{-1}]

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Chapter 8

Conclusions and Suggestions for Future Work

8.1 Conclusions

This thesis deals with different aspects of modeling, control and to a lesser extent, state estimation of continuous biotechnical processes. The research work has been motivated by the growing need to operate continuous bioprocesses in a cost-effective manner. With the growing economic pressure to increase product yield, improve product quality, and reduce production cost in biotechnology industry, the application of advanced control techniques has become of considerable interest and increasingly important. However, the development of modern control science in biotechnology industry has been hampered by two important obstacles: 1) lack of adequate sensors for process variables; 2) the complexity of biological systems which are not well understood. The aim of this thesis is to find more efficient control systems which provide quality assurance and economic incentives in the operation of continuous bioprocesses, and to cope with these two bottlenecks above mentioned. The main contributions of this thesis are summarized below:

Chapter 3 is the first major work to compare five control configurations for an idealized continuous stirred-tank bioreactor (CSTR), based on the controllability analysis with respect to the disturbance rejection. In particular, the partial disturbance gain, which is a frequency-domain controllability measure introduced by Skogestad and Wolff (1992), is generalized to the common case with more outputs uncontrolled. This work lays the foundation for control system design as presented in the thesis. Since the issue of disturbances has not been widely discussed in the literature on the controllability analysis for bioreactors, the use of the partial disturbance gain for the controllability analysis with respect to the disturbance rejection and for control structure selection is a new application in biotechnical process control. The main advantage with a controllability analysis is that it is independent of controller parameter tuning and makes detailed simulations unnecessary.

Chapter 4, Chapter 5, Chapter 6 and Chapter 7 are devoted to an extensive study of a special process, Propionibacteria fermentation.

Chapter 4 presents a preliminary study on Propionibacteria fermentation kinetics. From two batch fermentation experimental results, the Monod model is found to describe adequately the growth course of the microorganism

Propionibacterium shermanii, which is consistent with conclusions of Hettinga and Reinbold (1972a) and of Lee et al. (1974). Furthermore, some estimates for the parameters μ_m and K_s in the Monod model are obtained from these experimental results. This is the first work to treat the estimation of K_s corresponding to the complex medium yeast extract-peptone as limiting substrate. In the literature very little quantitative information is available about the fermentation kinetics of Propionibacteria growing on the complex medium yeast extract-peptone as limiting substrate.

Chapter 5 deals with the modeling work for an industrial *Propionibacterium shermanii* production in a continuous bioreactor with cross-flow filtration. The dynamic model is developed to describe the characteristics of this process, and its general features are extensively studied. The most interesting work is on the consideration of the Monod parameter K_s's influence on steady state behavior of process. Simulation results indicates that when K_s's value is high, following problems may arise: (1) washout more easily occurs; (2) more substrates need to be added into the reactor to obtain the desired high concentration of cell mass; (3) the problem with manipulated input constraints becomes considerable. Therefore, the knowledge about K_s is very important for good operation of this bioreactor.

Chapter 6 treats the on-line estimation of state variables in this process, by using an approach that incorporates material balance model with extended Kalman filter. The estimation model is developed that describes cell growth rate as a function of the dissolved CO_2 concentration and the base addition rate, which are more easily on-line measurable. Simulation results demonstrate the possibility to estimate the cell concentration and other state variables on-line from the available on-line measurements of dissolved CO_2 concentration and base addition rate, by applying the extended Kalman filter.

Chapter 7 has its focus on the final task for this industrial application, i.e. how to control this bioreactor properly and efficiently. At first by using simple frequency-dependent tools such as the relative gain array and the closed loop disturbance gain, the issues on the selection of manipulated inputs and how to pair the controlled outputs with manipulated inputs are discussed. Consequently, a 3×3 decentralized control configuration for this bioreactor

is proposed, and it is shown that if all three outputs were on-line measurable, then the 3×3 decentralized control system could work well to maintain all three outputs at their desired steady states subject to disturbances. Nevertheless, the key consideration is the possibility of applying partial control to this bioreactor. Based on the controllability analysis with respect to disturbance rejection by using the partial disturbance gain, a 1×1 partial control configuration is proposed. The main advantages with this control system lie in that (1) the main operation objective is achieved as the cell concentration is kept at its desired high value against disturbances; and (2) the practical implementation is possible as on-line measurement of lactic acid concentration is much easier than cell mass and yeast extract-peptone concentration.

An overall conclusion for Chapter 3 and Chapter 7 is that the partial disturbance gain is an effective tool for controllability analysis which is independent of the controller design and avoids time consuming on detailed time-response simulations.

8.2 Suggestions for Future Work

Although considerable emphasis has been placed on the control of batch and fed-batch bioreactors because of their prevalence in industry traditionally, continuous bioreactors are gaining considerable industrial importance as they provide better utilization of the equipment and the improved productivity. Therefore, there is a considerable scope for further development of advanced control strategies for continuous bioreactors. There remains a substantial number of outstanding research issues in continuous bioprocess control area, which have not been treated in full or even have not been considered in this thesis. In particular, the following topics should be investigated further:

8.2.1 Evaluation of control strategies for continuous bioreactors

As a continuation of the work presented in Chapter 3, on the evaluation of several control configurations for an idealized continuous bioreactor based on the Monod kinetics, it would be worthwhile to also consider other fermentation kinetics such as the substrate-inhibition kinetics or the Contois model (which takes into account the influence of cell concentration) (see Bastin and Dochain, 1990,

for more details). Furthermore, some more realistic process dynamic models, by including the metabolic product concentration M as an additional state variable, should be used in the controllability analysis work. Especially, Henson and Seborg (1992) claimed that for *productivity* (defined as DM) control, exact input-output-linearizing control employing the dilution rate D as the manipulated input provides excellent regulatory behavior; whereas input-output linearization with the feed substrate concentration as the manipulated input is problematic. One conclusion of Chapter 3 is that for *cell concentration* control, the control configuration with the feed substrate concentration as the manipulated input is superior to that using the dilution rate as the manipulated input when cell growth is substrate limited, which is in agreement with the results of Menawat and Balachander (1991) and of Agrawal and Lim (1984). Therefore, further investigations around this topic will probably reveal more peculiarities.

8.2.2 Continuation of the work on industrial Propionibacteria fermentation process

The second part of this thesis has focused on the Propionibacteria fermentation process. Regarding to this industrial process, at present there exist two limitations: (1) sufficient and reliable data of actual propionibacteria production process running in this continuous bioreactor with cross-flow filtration are not available yet; (2) the knowledge about the Propionibacteria fermentation kinetics with the complex substrate yeast extract-peptone as growth-limiting substrate are still lacking in the literature.

Fermentation kinetics

As shown in Chapter 5, the Monod parameter K_s has a large effect on the steady state behavior of process and then the knowledge about K_s is very important for good operation of this bioreactor. Off-line analysis of yeast extract-peptone, to determine which component of yeast extract-peptone really limits the cell growth, would therefore enhance the cell growth regulation over the precise nutrient specification of the culture medium.

On-line identification

Chapter 6 has investigated the possibility of using the on-line measurable environmental variables to estimate the unmeasured state variables in propionibacteria

fermentation process. Once the actual fermentation process data become available, a large effort needs to be done on extracting *a priori* knowledge of measurement noise and model uncertainty from real data analysis in order to implement the extended Kalman filter. An attractive feature of EKF is to provide combined state and parameter estimation, and then the Monod kinetic parameters μ_m and K_s and the yield coefficient $Y_{X/S}$ may also be estimated by treating them as additional state variables. Therefore, by incorporating off-line measurements on cell mass and other state variables, an adaptive on-line estimator can be developed. In the adaptive on-line estimator, the model parameters are updated whenever a new off-line measurement is available, and in between off-line samples these parameters are kept constant and used along with the on-line measurements to estimate the cell mass concentration and substrate concentrations.

Nonlinear control

It should be realized that bioprocesses can be highly nonlinear, which is of great importance in controller design. However, the issue of nonlinear control has not been addressed in this thesis because the nonlinear control approaches using "Exact Linearization techniques" heavily rely on the accuracy of the mathematical model of the fermentation kinetics which is not available yet regarding to the industrial process studied here. Nevertheless, improved control performance could be expected by explicitly taking into account process nonlinearities in the controller design. Further work should be done to fill this void.

Optimization

The high costs associated with many bioprocesses make optimization of bioreactor performance very desirable. Some initial work on this topic has been done (Zhao and Skogestad, 1994), which is not included in this thesis. There was not enough time to treat this in full. Most commonly for a continuous bioreactor, the index of performance for the optimization is the cell productivity. However, in this industrial process, the cell productivity $P = D_{\text{out}}X$ has no optimum with respect to the product flow rate D_{out} as $\frac{dP}{dD_{\text{out}}} < 0, \forall D_{\text{out}}$. It seems that in this bioreactor the good way to get as high cell productivity as possible is to increase the product flow rate D_{out} together with increasing the yeast extract-peptone feed flow rate D_{in1} (see Zhao and Skogestad, 1994, for details). Therefore, a more meaningful objective function (index of performance) needs to be defined

by taking into account not only cell productivity, but also cell yield coefficient and profit. Further investigation on the development of adaptive on-line optimization utilizing dynamic model identification would be most useful for cost-effective operation of this continuous bioreactor with cross-flow filtration.

At last, it should be kept in mind that, although new on-line measurement techniques offer the potential for a considerable improvement in bioprocess control, making more effective use of the measurements *already available* would prove extremely beneficial on real industrial bioprocesses. This aspect is the most basic consideration in control system development for bioprocess control. Research attempts in this thesis have been made towards this direction, as shown (1) in Chapter 6, two available secondary measurements, base addition rate and dissolved CO_2 concentration, are used to estimate the primary process variables, concentrations of cell mass and substrates (which can then be controlled); (2) in Chapter 7, a most efficient and practical 1×1 partial control configuration is proposed in which a more easily on-line measured process variable is selected as the controlled variable and the main operation objective is achieved.

Finally, it would be most desirable to implement on-line observers and controllers on this real industrial high concentration cultivation of propionibacteria in a continuous bioreactor with cross-flow filtration. This might reveal new issues of importance which were not covered here.

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