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Modelling and Control of Fermentation Processes

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Department of Automatic Control, Lund Institute of Technology

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Jan Peter Axelsson

Lund 1989

Dedicated to Franciskus of Assisi

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Modelling and Control of Fermentation Processes

Abstract

The possibilities for control of biotechnical processes using direct measurement of substrates and products, and what control structures that are appropriate have been investigated. Baker's yeast served as a model organism for study of control of cell growth, and of control of product formation. Experiments were done on a laboratory scale.

Fed-batch cultivation of yeast was controlled based on ethanol measurement in the broth. The yeast is very sensitive to small changes in their reactor environment. The metabolism changes in case of over-feeding to ethanol production, and in case of under-feeding ethanol is consumed. The dynamics of switch of metabolism was studied in identification experiments. The results were compared with what was expected from the underlying stoichiometry based on the bottle-neck principle. The results from identification were also useful for design of the control system. The main reason for control is to track the drastic growth in feed demand during a cultivation. Uncertainty in this growth was considered the main disturbance, while variations in process dynamics were regarded of minor importance. An approximate internal model of the feed demand was found to facilitate design of a robust control system using a low-order regulator with fixed parameters.

Continuous ethanol fermentation from sucrose was controlled using information from measurements of substrate and product. A tank and a tube reactor were used in combination. The yeast was immobilized in alginate gel. Different control structures were tested at the process. Control of the continuous tank reactor was further analysed. The non-linear character of the tank dynamics implies that start-up and large disturbances from the stationary state, call for other control strategies than elimination of small disturbances and change of set-point. A time optimal formulation gives, in fact, reversed control actions for small and large disturbances. The problem was solved using dynamic programming and a bang-bang control law was obtained. The control problem was also analysed using differential geometric concepts.

Key words
Baker's yeast, Biosensors, Identification, Internal model principle, Non-linear control

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Preface

The experimental work in this thesis has been carried out in close cooperation with the Department of Biotechnology. It has been exciting, but hard, to work in an interdisciplinary project like this. The stimulating part has been to enter the field of biotechnology with a different background than is traditional. A control engineering point of view may certainly help to understand the intricacies of cellular metabolism as well as how to control a bio-reactor.

This thesis is mainly written for a person with a background in control engineering at the graduate level. Therefore a chapter on elementary yeast biology is included and elementary facts on reactors are also briefly reviewed. However, I hope that parts of the thesis will be read by biochemists and microbiologists as well, and stimulate further research on cellular dynamics. I have, actually, experienced a substantial interest in process control from the biotechnical community.

Acknowledgement

I would like to thank Professor Karl Johan Åström who has entrusted me to work in this area and who has given his support. Docent Per Hagander has been my supervisor. I am grateful for his sincere interest in this work and for his painstaking but fruitful criticism of my writing.

A large part of the work has been carried out as an interdisciplinary project between the Departments of Biotechnology and Automatic Control. There has been several people involved in the experimental work and without their effort little had been done. The project was initiated by Professor Bo Mattiasson and Docent Per Hagander. It was sponsored by STU, the Swedish Board for Technical Development, under contracts 82-3359 and 82-3494.

I would like to thank my colleagues Carl Fredrik Mandenius and Olle Holst for fruitful collaboration over many years. Olle has contributed with his microbiological knowledge and experience. Carl Fredrik developed and refined the ethanol sensor. Several papers are coauthored, and Chapters 4 and 7 are to a certain extent based on (Axelsson *et al*, 1988) and (Mandenius *et al*, 1987), respectively. I am indebted to Lena Nielsen and Björn Rosén for excellent technical assistance and for willingness to follow the experiments far beyond regular working hours. I would like to thank Günther Held for service of the equipment, often with short notice. A special thank to Leif Andersson for support of the software to the computer and for advice concerning real-time programming.

I would like to thank all my colleagues at the department. I am indebted to Bo Bernhardsson, Rolf Johansson, Bengt Mårtensson, Bernt Nilsson and Professor Björn Wittenmark for valuable comments on the manuscript, and to Britt-Marie Mårtensson who skilfully prepared the figures.

Finally, my thoughts go to my dear parents, and I would like to thank them for encouragement and support.

1

Introduction

The inspiration of this work is a fascination of the living cell and its multitude of controlled chemical reactions. It is a challenge to try to understand the dynamical aspects of cellular metabolism and growth. The industrial use of microorganisms calls for a better understanding of how to maintain favourable conditions for the cells in a fermentor. Feedback control may be an important technique to improve process intensity.

On-line measurement of important variables are difficult in biotechnical processes. Therefore various techniques to calculate these variables from others more easily measured, have attracted research interest. The complexity of chemical processes makes estimation from such indirect measurements more difficult and uncertain compared to estimation of, for instance, velocity and acceleration in mechanical systems. Control based on such indirect measurements are therefore less certain to maintain process conditions.

This thesis is an investigation of the possibilities for process control using direct measurement of substrate and product concentrations, and what control structures that are appropriate. Experiments have been carried out on a laboratory scale and new on-line measurement techniques were employed. Yeast has served as a model organism for study of control of cell growth, and of control of product formation. The work is to a large extent experimental in nature. However, a few observations have led to theoretical considerations of a more general interest.

Outline of the thesis

The thesis consists of three parts: cellular dynamics of yeast, fed-batch cultivation, and continuous ethanol production.

The first part is a critical literature study. In Chapter 2 a brief background

is given in yeast biology. Focus is on aspects relevant for understanding cellular dynamics. In Chapter 3 the biochemistry of sugar metabolism is quantified and the stoichiometry for different metabolic paths are given. This model has emerged from chemostat experiments and thus reflects stationary states of the cell culture. The relevance for transient conditions is discussed.

The next three chapters are devoted to fed-batch cultivation of yeast. This part of the thesis is backed up by a large experimental effort. Chapter 4 gives a motivation for using feedback control of fed-batch production. An evaluation is also done of our own controlled cultivations in terms of yield and growth rate. Chapters 5 and 6 deal with the actual design of the control systems. A dynamical model is developed for the process. The model is confirmed by several identification experiments done at different cell concentrations. The results are related to the stoichiometry presented in Chapter 3. Differences between cultivations are also characterized and main process disturbances are identified. The design of the control system is presented in Chapter 6. Limitations of conventional PID control are discussed. The major control difficulty is identified as to track the drastic growth in the feed demand. This view leads to a regulator with an observer for the feed demand. The robustness properties of such a regulator are analysed and compared with PID control.

The last part of the thesis deals with continuous production of ethanol using yeast. In Chapter 7 the reactor configuration is discussed. The presentation of the experimental part is focused on observed dynamics of substrate and product in a tank- and in a tube reactor. The non-linear character of the tank dynamics complicates control. Physical insight indicates that reversed control actions may be appropriate for large disturbances compared to compensation of smaller disturbances. In Chapter 8 these ideas of reversed control actions are further investigated. A time-optimal control problem is formulated and solved. The problem is also approached by the method of exact linearization.

Finally, conclusions and suggestions for further research are given in Chapter 9. Details of the different experimental set-ups and the Simnon code for the model of the yeast culture are given in two appendices.

2

Elementary Yeast Biology

In the living yeast cell a vast number of chemical reactions are integrated to a functioning whole. Part of this inherent complexity is revealed in the interactions with the environment. The cell needs both material and energy for maintenance and growth. There is a constant exchange of different compounds between the cell and the surrounding media. The yeast cell has also the capability to accumulate and store reserve material. It can adapt to various conditions after a lag phase of a few hours.

When yeast is cultivated on an industrial scale or used for production of certain compounds, it is of interest to maintain favourable conditions for the cells. Process control faces both the task to regulate the environment of the cell and to adjust for lag phases of cell adaptation. This latter part makes control of biotechnical processes more demanding than other chemical processes that exploit catalysts for example.

The yeast cell is well known in comparison with other microorganisms. The reference work on yeast is (Rose and Harrison, 1971). A short and up to date account is (Berry, 1982). This chapter gives a brief overview of yeast biology that is believed to be relevant for process control. Emphasis are on facts that may have importance for understanding dynamics of the yeast culture. In Section 2.1 major parts of the yeast cell are described. An idea of orders of magnitude is also given. Section 2.2 describes central parts of sugar metabolism. Then follows in Section 2.3 a background to why substrate control is important for cultivation of yeast cells. The background for high rate ethanol production and effects of immobilization of the yeast cells on productivity are given in Section 2.4.

2.1 A brief description of the cell

Yeast is a large and complex microorganism that belongs to the class of Fungi. It has about a thousand times larger volume than a bacteria and it has a cell nucleus, like all higher organisms. It reproduces generally by budding and cell division, but sexual reproduction may also occur under certain circumstances. In Figure 2.1 is shown a drawing of a typical yeast cell.

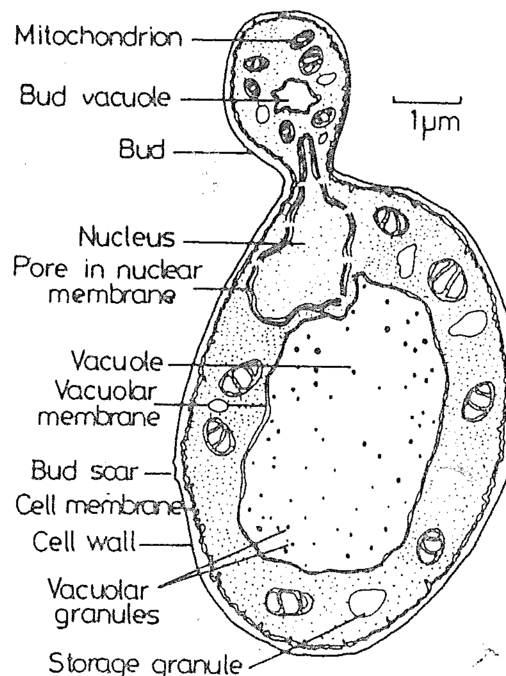


Figure 2.1 Section through typical yeast cell showing the main features of the cell and their distribution. Reproduced from Webster, 1980, *Introduction to Fungi*, p. 273. Cambridge University Press.

Cell morphology

The yeast cell is a robust organism in several aspects. The cell wall is a rigid structure that constitutes 25 % of the cell dry weight. It not only protects the cell but also contains several enzymes. The inside of the cell has a certain structure, as seen in Figure 2.1. The cell contains a cell nucleus where the DNA is located. There is a number of different functional units, organelles. There are up to 50 mitochondria in a yeast cell and there an important part of the cell metabolism takes place. Other parts of the metabolism takes place in the cytosol which is the wide spread dotted area in Figure 2.1. Sugars and lipids are stored in storage granules. A large part of the cell interior is made up of a vacuole. The function of the vacuole is not clear. It may serve as a storage reservoir for nutrients

and certain enzymes. There is also a hypothesis that wastes and toxic products accumulate in the vacuole.

Orders of magnitude

It is always a good idea to have an approximate notion of orders of magnitude. The yeast cell has a diameter of 5-8 μm. This one or two orders of magnitude smaller than a typical air buble. The volume of the yeast cell is about 10^{-12} L. The internal concentration of intermediate metabolic compounds in the glycolysis in a typical cell are in the range 50-5000 μ M (Bailey and Ollis, 1986), i. e. about $10^7 - 10^9$ molecules in a yeast cell. A typical flow of glucose during a cultivation is about $10^4 - 10^5$ molecules per yeast cell each second. This gives an approximate turn over time of $10^1 - 10^3$ s. These crude calculations should be compared with the estimated turn over of ATP in *E. coli*, a bacteria, which is a few seconds (Bailey and Ollis, 1986).

2.2 Sugar metabolism

Sugar is used by the cell both as a carbon source and as an energy resource. The sugar metabolism consists of many steps and is spread out over large parts of the cell. There are many internal control mechanisms that integrate the different parts. The production of ethanol can be understood in terms of an overflow of an intermediate metabolite in the system. This state affects cell growth and much of the metabolism.

The metabolism of sugar can be divided into two parts. Actually, there is also a pre-stage on the outside of the cell where disaccharides are cleaved to monosaccharides. Inside the cell, in the cytosol, the glucose is halfway metabolized to pyruvate essentially. This part of the metabolism is called glycolysis. Pyruvate is an intermediate metabolite with a key role for synthesis. About half of the pyruvate is used for building cell material. The other half is taken up by certain organelles, mitochondria, where most of it is further metabolized into water and carbondioxide respiration. This part is the citric acid cycle. The net effect is energy stored in ATP, reducing equivalents NAD, and actually some of the intermediates, like malate and oxalacetate, are precursors for synthesis.

2.3 Balanced sugar supply and critical growth rate

The ethanol production can be understood as an overflow reaction. The glycolysis in the cytosol produces more pyruvate than the respiratory system in the mitochondria can metabolize. In this case pyruvate is reduced to ethanol. On the other hand, if there is ethanol and too little glucose available to the cell, ethanol can be converted and metabolized by the mitochondria. In case of excess glucose and ethanol in the broth, glucose is preferentially metabolized.

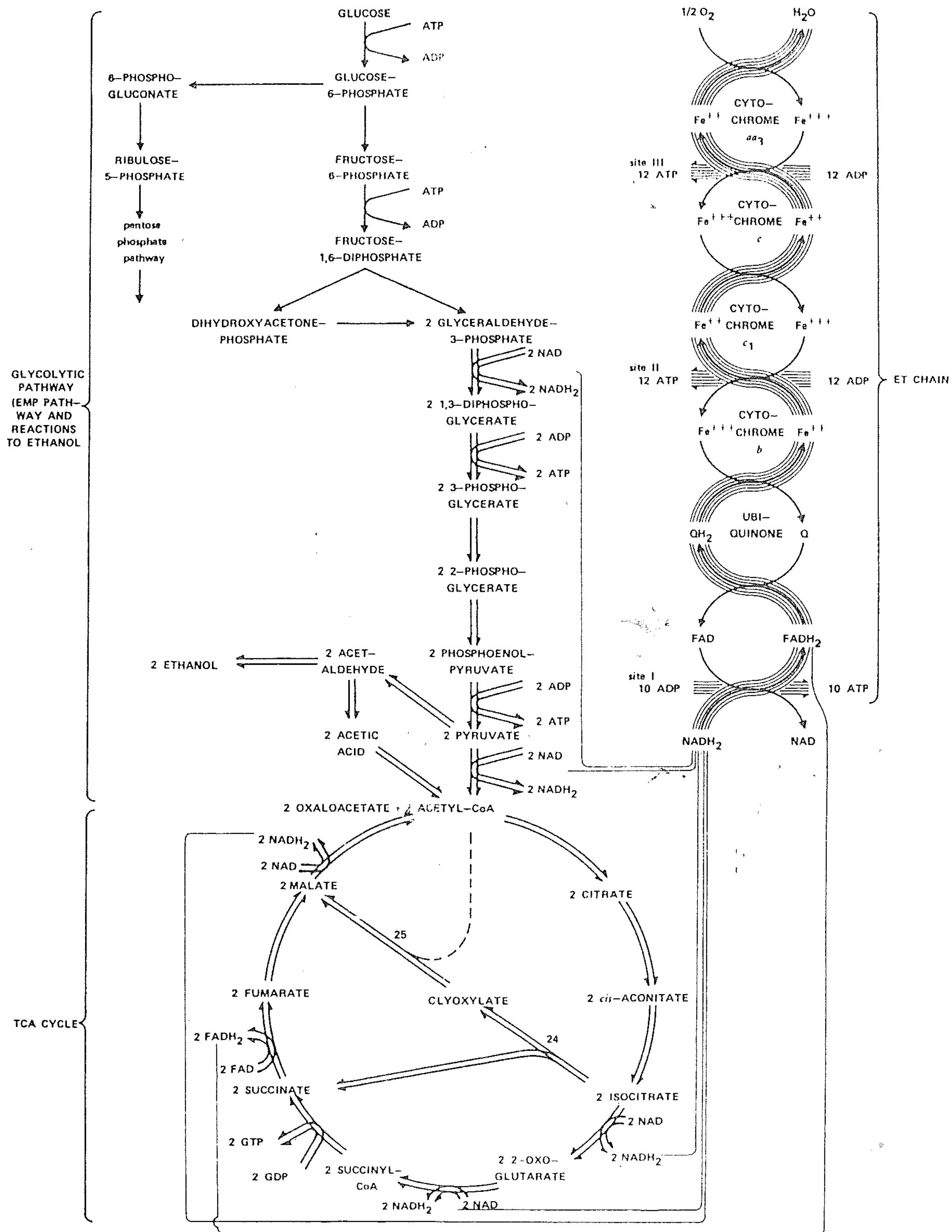


Figure 2.2 Schematic view of the glycolys, citric acid cycle and the glyoxylate cycle. The electron transfer chain is also shown. Reproduced from (Oura, 1972).

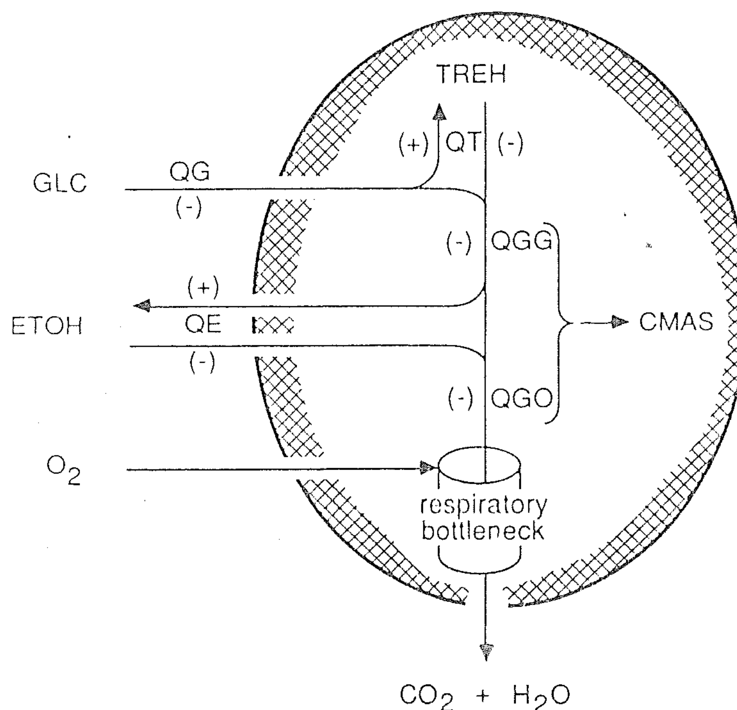


Figure 2.3 Structure of the metabolic flow of glucose and ethanol with emphasis on the bottle-neck principle. GLC=glucose; ETOH=ethanol; TREH=trehalose; CMAS=cell mass. The flows of metabolites are denoted with QG, etc. Reproduced from (Strässle, 1988).

The conversion of ethanol to acetate is done in the cytosol and is further oxidized in the citric acid cycle and glyoxylate cycle in the mitochondria and the glyoxsomes, respectively. The growth on ethanol requires the production of many precursors for synthesis that the glycolysis otherwise provides when growth is on glucose. In order to produce these precursors the glycolysis is essentially run backwards. It is referred to as the gluconeogenic pathway.

There are several theories for where the rate limiting step is located. For our purpose, it suffices to regard it as a limited respiratory capacity (Käppeli and Sonnleitner, 1986). Under certain circumstances glucose is stored within the cell in the form of the polysaccharides glycogen and trehalose. At low cell growth there is a pronounced interaction with this storage (Heinzle *et al*, 1982; Kätterer *et al*, 1986); Another storage is the lipid fraction of the cell that may play a role in glucose metabolism. Altogether these storages can account for up to 30 % of the cell mass. Uncontrolled physical storages, may be more important to understanding yeast metabolism dynamics than biochemically controlled glycogen and lipid storages. The internal pool sizes are important to consider. For example, the pool size of ATP is small. In *E. coli* it would only last for two seconds if the substrate supply ceased (Bailey and Ollis, 1986). Intracellular carbondioxide

accumulation is discussed in (Barford and Hall, 1979). The vacuole of the cell might play a role as a short time reservoir of substrates and products.

During growth, the individual yeast cells change their metabolism. There are two main phases, single cell phase and budding phase. The single cell phase is characterized by respiration and volumetric growth at a rate depending on the substrate concentration. The budding phase has constant length, metabolism is fast, and ethanol is produced and excreted from the cell (Meyenburg, 1969). For a population of cells, synchronization of cell growth disappears after about one or two generations. Therefore it is reasonable to disregard the cyclic changes of metabolism on a macroscopic level. However, recently there are reports that show spontanuous synchronization effects in continuous cultures (Sonnleitner *et al*, 1986; Parulekar *et al*, 1986; Strässle, 1988).

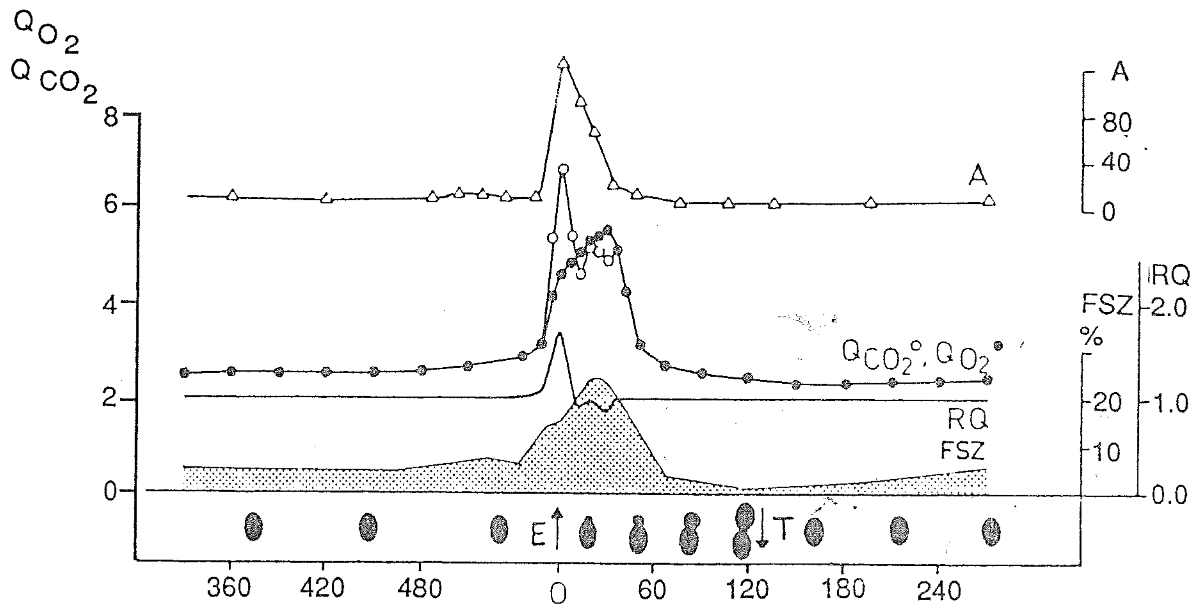


Figure 2.4 Recording from a synchronous culture showing variations in the metabolism during different stages of the cell cycle. At E the budding phase starts and at T the daughter cells leave the mother cells. A=ethanol [mg/L]; FSZ=ratio of budding cells [%]; The time scale is in min. Reproduced from (Meyenburg, 1969).

2.4 Excessive sugar supply

Under anaerobic conditions a yeast cell culture partially metabolizes glucose to ethanol. During this process the cell extract energy. Such microbiological reactions where energy is extracted without consumption of oxygen are called fermentations (Pasteur, 1861).

The yeast cell has a large capacity to break down sugar to ethanol. However, the cell gets only about 5-10% of the available energy in sugar this way. It is a

way for the cell to quickly obtain energy. This is indicated in Figure 2.4, where it is seen that the cells produced ethanol during the energy demanding budding phase, despite the fact that oxygen was available. At high sugar concentrations the yeast cell growth rate increases far beyond the capacity of complete oxidation of glucose, and large amount of ethanol are produced despite aerobic conditions. Similarities with tumour growth has been discussed (Crabtree, 1929).

When yeast cells are used for ethanol production cell growth is of minor interest, and is actually more of a problem. If the medium to the cells do not contain nitrogen the cells do not grow. However, they still produce ethanol. Immobilization of yeast cells is an important technique in process design (Núñez and Lema, 1987). The yeast cells are trapped in gelatin gel and higher cell concentrations are possible in the reactor. When the yeast cells are immobilized growth is inhibited and the ethanol productivity is improved. There are also different ways to manipulate the microenvironment of the cells to enhance ethanol production (Larsson *et al*, 1988).

3

Quantitative Studies of Yeast

There is a considerable amount of quantitative knowledge of metabolic flows in yeast, and how these flows are related to cell growth. This knowledge has been obtained mostly from chemostat experiments where yeast cultures have been grown at different growth rates under precise conditions. From such experiments relations are obtained between substrate and oxygen uptake rates, product formation rates and cell growth, under stationary conditions. These relations are often expressed as stoichiometric formulas but do not actually convey much information about the underlying multitude of reactions. From a control engineering view point it is important to understand and account for these basic relations, and especially to try to extend the understanding to transient behaviour of cell cultures. Also a good idea of what is more uncertain about cellular behaviour may help to make a good control system design.

This chapter starts in Section 3.1 with a short discussion on the possibilities to monitor the yeast metabolism. In Section 3.2 the current understanding of different modes of yeast metabolism is described. The experimental technique is briefly reviewed as well. In Section 3.3 the implication of the stationary model on transient behaviour is examined. The relation beween uptake rates and concentrations is discussed in Section 3.4.

3.1 Monitoring of the glucose metabolism

In order to monitor the glucose metabolism of a yeast culture, the ideal metabolite to measure would be the intracellular concentration of pyruvate. Today this is

not feasible. However, the imbalance of glycolysis and respiration quickly leads to a change in the intracellular concentration of reducing equivalents, NAD. These changes can actually be monitored using fluorescence techniques (Meyer and Beyler, 1984; Scheper and Schügerl, 1986). The energy or ATP demand in the cell, controls and integrates to a large extent the cell metabolism. There are some off-line studies made (Meyenburg, 1969) but these are difficult to do and there is no on-line technique available today.

Extracellular measurement techniques are valuable. The fast breakdown of pyruvate to ethanol combined with the fact that the ethanol is excreted from the cell, makes measurement in the broth of this compound informative. Another variable reflecting the metabolic state is the carbon dioxide production rate. This is due to the fact that carbon dioxide is produced when pyruvate is metabolized to ethanol. Usually the measurement of the rate of carbondioxide production is normalized with respect to the measured oxygen uptake rate, i. e. the respiratory quotient or RQ. Ideally, RQ is proportional to the rate of ethanol production/consumption.

The RQ measurement is a wide spread technique to monitor cell cultures. In the research laboratories expensive mass spectrometers have been used to get further information from the exhaust gas from the fermentors (Pungor *et al*, 1983). In this way different volatiles like ethanol, acetaldehyd etc, can be monitored as well as the respiratory quotient. However, there are a number of difficulties with exhaust gas analysis in general. The mass transfer between the liquid phase and the gas phase is influenced by a number of factors, e. g. humidity of the gas phase, pressure and pH of the broth (Dekkers, 1982). A more direct technique is the use of semipermeable membranes to extract volatiles from the broth for analysis (Kobayashi *et al*, 1979). Fouling of the membrane is here a negligible problem. The gas can then be analysed with various techniques, e. g. mass spectrometer (Punger *et al*, 1983), gas chromatography (Pons *et al*, 1988), or cheap semiconducters (Yamané *et al*, 1981; Mandenius and Mattiasson, 1983). These techniques have various degrees of specificity and response time.

Substrate and cell concentration are two fundamental variables that are difficult to measure on-line. Off-line analysis are in many cases the only source of information. However, it is difficult to avoid systematic errors in samples taken from the reactor. The cells consume the substrate in the sample within 1-30 s. The internal status of the cell is even more difficult to get an idea of from off-line analysis. However, certain structural changes like enzyme and RNA contents vary in a slower time scale.

3.2 Stoichiometric quantification

Glucose is used by the cell both as an energy source and as a source of building material. There are several attempts to formulate a quantitative description of glucose metabolism and cell growth. An important choice when making a

model is the degree of details of the metabolism that are accounted for. Here the approach developed at ETH is followed (Käppeli and Sonnleitner, 1986). The model is based on external measurable quantities only. There are several other models that relate to the internal flow of ATP and NAD in different ways (Bailey and Ollis, 1986). The model discussed here is valid over a large range of growth rates. However, cellular maintenance is not accounted for. At low growth rates this becomes important to consider and a more complex model is called for. The experimental background is first briefly reviewed.

Chemostat experiments

The chemostat, or continuous stirred tank reactor, is a valuable tool in the research of microbial cell metabolism (Pirt, 1975). The yeast has been studied in this way by many research groups. The research has been concentrated on understanding the stationary states.

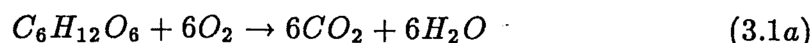
The chemostat gives the possibility to study microorganisms at different rates of cell growth (Monod, 1950), compared to batch experiments. The cells are grown under conditions where the substrate is the limiting factor. Let the feed contain the limiting substrate. Then the feed flow rate determines the rate of cell growth. In equilibrium the cell growth rate equals the dilution rate, i. e. the flow rate through the reactor per volume unit. In case the cell culture grows faster the substrate concentration will decrease and the growth rate goes down, and vice versa. Thus, the equilibrium is stable, provided the growth rate increases with the substrate concentration.

It takes time to reach steady state. The time constant is inversely proportional to the dilution rate, and it is necessary to wait 3-5 time constants. This means for example, that in order to study a cell culture at a growth rate 0.10 h^{-1} it is appropriate to wait a day or two for the steady state. To go through a number of dilution rates could thus easily take a couple of weeks and such long experiment periods call for stable equipment to keep everything else constant. An important questions is also the genetic stability of the microorganism under study. However, in case of yeast this is not a problem.

The classical stoichiometry

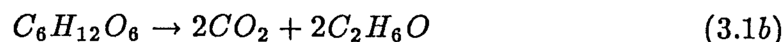
The yeast cells can grow under different conditions. The classical stoichiometry for respirative glucose or ethanol breakdown and for fermentative ethanol formation separates these reactions from cell growth. The stoichiometry for the reactions are

Respiratory growth on glucose:



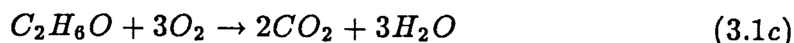
$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad (3.1a)$$

Fermentative growth on glucose:



$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_6O \qquad (3.1b)$$

Respiratory growth on ethanol:



$$C_2H_6O + 3O_2 \rightarrow 2CO_2 + 3H_2O \tag{3.1c}$$

For a long time this was the prevailing view of yeast glucose metabolism. However, refinement of the exhaust gas analysis made it difficult to disregard results from chemostat experiments that showed RQ values slightly different from what is expected from this model. During respirative growth on glucose this model predicts an RQ value 1.00, but from experiments 1.08 was found. For growth on ethanol an RQ of 0.67 is expected but careful measurements only give 0.42.

Account of cell mass formation on the stoichiometry

The classical stoichiometry gives a slight error in the prediction of the respiratory quotient for the different growth conditions. A better model should include formation of cell mass and also account for the nitrogen source, because they contribute to the redox balance (Barford and Hall, 1979). A simplifying fact is that despite variations in growth conditions the cell mass composition remains fairly constant. The modified stoichiometry for the three pathways is given below.

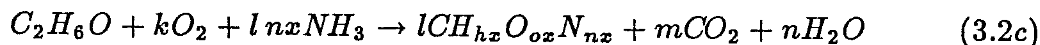
Respiratory growth on glucose:

$$C_6H_{12}O_6 + aO_2 + b\,nxNH_3 \rightarrow bCH_{hx}O_{ox}N_{nx} + cCO_2 + dH_2O \tag{3.2a}$$

Fermentative growth on glucose:

$$C_6H_{12}O_6 + g\,nxNH_3 \rightarrow gCH_{hx}O_{ox}N_{nx} + hCO_2 + iH_2O + jC_2H_6O \tag{3.2b}$$

Respiratory growth on ethanol:



$$C_2H_6O + kO_2 + l\,nxNH_3 \rightarrow lCH_{hx}O_{ox}N_{nx} + mCO_2 + nH_2O \tag{3.2c}$$

The next step is to determine the stoichiometric coefficients. Each reaction can be considered separately. Elemental balances for C, H, O and N give rise to a system of equations. There are three independent equations and four unknowns; hence a measurement must be done in order to determine the system of equations. One choice is to measure the cell yield. Sensitivity evaluation shows good behaviour.

Analysis of the cell mass gives (Sonnleitner and Käppeli, 1986)

$$hx = 1.79 \qquad ox = 0.57 \qquad nx = 0.15 \tag{3.3}$$

Measured yields gives (Sonnleitner and Käppeli, 1986)

$$b = 3.50 \qquad g = 0.36 \qquad l = 1.32 \tag{3.4}$$

Solution of each system of elemental equations

$$
\begin{array}{llll}
a = 2.33 & (b = 3.50) & c = 2.50 & d = 3.66 \\
(g = 0.36) & h = 1.89 & i = 0.14 & j = 1.88 \\
k = 1.61 & (l = 1.32) & m = 0.68 & n = 2.12
\end{array}
\tag{3.5}
$$

An alternative to measurement of cell yield is measurement of the respiratory quotient, to determine each system of equations. However, sensitivity analysis shows that in this way the solution becomes very sensitive to errors in the RQ measurement as well as in the analysis of the cell mass composition. This sensitivity is especially pronounced in solving the equations for respirative growth on glucose. This difficulty is discussed at length in (Stephanopoulos *et al*, 1984).

In the elemental equations, the nitrogen balance does not give any new information. This is due to the fact that the single nitrogen source is converted completely into biomass. However, the nitrogen source indirectly gives a contribution to the redox balance through the other atoms. In the case of ammonia, the hydrogen atoms affects the overall hydrogen balance. This is discussed thoroughly in (Barford and Hall, 1979; Roels, 1980; Bruineberg *et al*, 1983) A different nitrogen source would alter the elemental equations and could thus change all parameters (3.5).

Switch of metabolism: the bottle-neck concept

So far, the yeast metabolism and growth have been described for different simplified cultivation conditions. A more complex situation arises, for instance, when both glucose and ethanol are available. In these more complicated situations the cell growth rate μ can be described as a linear combination of the different pathways (3.2a-c). Each path contributes in proportion to the reaction rate, q_G^{resp}, q_G^{ferm} and q_E^{resp} respectively. Denote the corresponding yields (3.4) by Y_G^{resp}, Y_G^{ferm} and Y_E^{resp}, which gives

$$\mu = Y_G^{resp} q_G^{resp} + Y_G^{ferm} q_G^{ferm} + Y_E^{resp} q_E^{resp} \tag{3.6}$$

The switch between the different pathways is thought to be governed by the respiratory capacity $q_{O_2}^{lim}$. If the glucose uptake rate is low, growth is respiratory. In this metabolic state the yeast culture can consume both glucose (3.2a) and ethanol (3.2c) up to the limitation

$$a q_G^{resp} + k q_E^{resp} < q_{O_2}^{lim} \tag{3.7}$$

However, glucose is preferably metabolized. The ethanol uptake rate q_E^{resp} is governed by the remaining oxidative capacity. At higher glucose flux rates, when

$$a q_G > q_{O_2}^{lim} \tag{3.8}$$

ethanol is produced. The growth is said to be respiro-fermentative or oxido-reductive, i.e. a certain part of the glucose q_G^{resp} is oxidized completly by (3.2a) and the overflow q_G^{ferm} is reduced to ethanol, through (3.2b).

The bottle-neck principle implies that ethanol is not simultaneously produced and consumed (Sonnleitner and Käppeli, 1986). However, in a large reactor it is difficult to maintain homogeneous conditions and a situation may arise where ethanol is produced by some yeast cells and consumed by others, giving a significant decrease in the yield. This situation is discussed in (Rajab *et al*, 1984; Sweere *et al*, 1988).

3.3 Stoichiometry during transients

The possibilities to extend the results from stationary conditions, as in the chemostat experiments, to conditions where the substrate concentration changes is here discussed. A "stoichiometric gain" will be derived for small variations in the glucose uptake rate to variations in the production/consumption of ethanol. Transient phenomena have in general been neglected until recently (Kätterer *et al*, 1986; Sweere *et al*, 1988). The advent of new on-line measurement techniques pave the way for a better understanding of dynamics of cell cultures.

Physical and microbiological background

Transients in measurement signals are more difficult to interpret than stationary levels. There are several aspects to consider, both microbiological and physical.

The internal pools of metabolites are small in comparison with the turn over. For instance, the ATP-pool would only last for a few seconds if the substrate supply ceased. The same order of magnitude holds for the NAD-pool. The coupling between metabolism and synthesis of cell material is complex and there are several regulating functions. Changes in the cell growth rate varies in the time-scale of half an hour. The internal storage of glucose appears to operate in the same time scale. It is worth considering that a glucose storage of typically 30 % of the cell weight would last for about 15-30 min at the critical growth rate, if the glucose supply was shut off.

A critical study shows that mass transfer between the cell culture and the broth accounts for some dynamics. Further, the mixing properties of the reactor are important to consider when transients of a cell culture are studied (Kätterer *et al*, 1986). High performance bioreactors have been designed for research purpose, with a mixing time of less than 1 s (Sonnleitner and Fiechter, 1988).

The measurement technique is important to scrutinize in order to avoid systematic errors in transient recordings. Respiratory quotient suffers from several shortcomings for dynamical studies of cell cultures. Most evident is that the volume of the head gas phase introduce additional dynamics. The mass transfer between the liquid phase and the gas phase in the broth is complex (Dekkers,

1982). The solubility of oxygen in the broth is low and therefore measurement of the oxygen consumption of the culture should be possible to track even during transients. However, the solubility of the carbondioxide cannot be neglected. The broth contains a certain amount of carbondioxide and even the cells may contain or bind some carbondioxide temporarily (Barford and Hall, 1979). The situation is complicated by the fact that the solubility of carbondioxide is dependent on the *pH* (Stephanopoulos *et al*, 1984). At the cellular level, there are certain metabolic states that give an increase in both carbondioxide production and oxygen consumption (Pons *et al*, 1986). Such phenomena are hidden in the RQ signal. The technique of extracting volatiles by a semipermeable membrane offers a more direct way to monitor the yeast culture. The diffusion over the membrane introduces a time constant that can be made short, and there are less factors that may give systematic errors, especially during transients.

Stoichiometric equations applied to on-line estimations

In industrial production interesting parameters are yield and growth rate. Lack of direct sensors for substrate, product and biomass has stimulated research on using indirect measurements to monitor important variables. Knowledge of the stoichiometric relations in combination with measurement of respiratory quotient has been used to get an estimate of these variables (Cooney *et al*, 1977; Zabriske and Humphrey, 1978). More advanced filtering techniques were used in (Dekkers, 1982). A critical review of the difficulties with estimation of the status of the cell culture from these measurements are given in (Stephanopoulos *et al*, 1984). For the discussion here, it is noted that the stoichiometry for stationary cell conditions has been used in these applications without much discussion about the validity during transient conditions.

Derivation of a stoichiometric gain

Provided the stoichiometry (3.1) or (3.2) is valid also during transient conditions, it is possible to determine how sensitive an indicator ethanol is, to deviations in the glucose feed rate around a feed rate that corresponds to the respiratory capacity. This sensitivity will be referred to as the "stoichiometric gain". It is closely related to the process gain that is experimentally determined in Chapter 5. The process gain plays a crucial role in the design of a control system. It is of interest to know its value and how it depends on other process parameters. Therefore it is very interesting to know the underlying stoichiometry that determines the gain.

From the stoichiometric equations (3.2), a relation between the glucose uptake rate q_G and the ethanol formation rate q_E can be derived, based on the following assumptions. The switch in metabolism between ethanol production and consumption is viewed as an overflow reaction governed by the respiratory capacity $q_{O_2}^{lim}$, as discussed in Section 3.2. Furthermore, in the case of over-feeding with glucose, the stoichiometry is a sum of equations (3.2a) and (3.2b). In the

case of under-feeding with glucose, ethanol is consumed by the remaining respiratory capacity and the stoichiometry is a sum of equations (3.2a) and (3.2c), i. e., reaction (3.2c) momentarily absorbs all the respiratory capacity left over from the reaction (3.2a)

$$q_E = \begin{cases} j q_G^{(b)} = j(q_G - q_G^{(a)}) & = j(q_G - \frac{1}{a} q_{O_2}^{lim}), \quad q_E > 0 \\ -\frac{1}{k} q_{O_2}^{(c)} = \frac{1}{k}(a q_G^{(a)} - q_{O_2}^{lim}) & = \frac{a}{k}(q_G - \frac{1}{a} q_{O_2}^{lim}), \quad q_E < 0 \end{cases} \tag{3.9}$$

Introduce the concept of a stoichiometric gain κ and let $\Delta q_G = q_G - q_{O_2}^{lim}/a$,

$$q_E = \kappa \Delta q_G \tag{3.10}$$

where

$$\kappa = \begin{cases} j \approx 1.9, & q_E > 0 \\ \frac{a}{k} \approx 1.5, & q_E < 0 \end{cases} \tag{3.11}$$

In a similar way a stoichiometric gain can be derived for the sensitivity of the respiratory quotient to variations in the glucose uptake rate Δq_G. Introduce the notation $\Delta RQ = RQ - RQ_{crit}$ where $RQ_{crit} = c/a$

$$\Delta RQ = \kappa_{RQ} \Delta q_G \tag{3.12}$$

where

$$\kappa_{RQ} = \begin{cases} h/q_{O_2}^{lim} \approx 1.9/q_{O_2}^{lim}, & q_E > 0 \\ (m\frac{a}{k} - c)/q_{O_2}^{lim} \approx 1.5/q_{O_2}^{lim}, & q_E < 0 \end{cases} \tag{3.13}$$

Note that in these derived gains there are a ±10% variation between under- and over-feeding. In practice, however, this difference is likely to be evened out, since some cells are underfed and other cells are overfed, due to a certain variation of $q_{O_2}^{lim}$ in the culture. In a large reactor inevitable variations in the substrate and dissolved oxygen concentrations may complicate the picture.

Sources of variations in the gain

There are several factors that may have an influence on the value of the stoichiometric gain κ. A fundamental question is whether the stoichiometric (3.2) model is valid for rapid changes in the glucose uptake rate. One reasonable possibility is that for such rapid changes the metabolism is decoupled from cell growth and that the classical stoichiometry (3.1) is more appropriate. In Table 3.1 the values of the stoichiometric gain are compared for the two different models. It is found that the gain is slightly higher for the model that does not account for cell growth.

Table 3.1 Values of the stoichiometric gain κ for different models.

Model	κ^+	κ^-
3.1	2.00	2.00
3.2	1.87	1.45

The influence of lack of oxygen on the stoichiometric gain is most important to understand, since it is difficult to maintain high dissolved oxygen concentrations throughout a cultivation on an industrial scale. Lack of oxygen could be thought of as a lowered value of $q_{O_2}^{lim}$. The result (3.9) then implies that the glucose feed rate must be decreased, correspondingly. However, from this stoichiometric model there are no reasons to believe that the gain κ will change.

Variations in the kind of nitrogen supply are also realistic in industrial production of yeast. The nitrogen source is generally ammonia, NH_3. However, the yeast is often grown on molasses and it contains nitrogen in the form of amino acids, especially glutamat. During certain periods of a cultivation glutamat may be an important nitrogen source (Hill and Thomel, 1982). Under such conditions the stoichiometry may be altered, as mentioned in Section 3.2, and the gain κ changed correspondingly. In Table 3.2 is shown the corresponding values of κ. In practice the nitrogen source would be a combination, giving a κ in between.

Table 3.2 Influence of the nitrogen source on the stoichiometric gain κ.

Nitrogen source	κ^+	κ^-
NH_3	1.87	1.45
HNO_3	1.84	1.05
glutamat	1.95	2.83

Variations in the nitrogen supply may affect the cell metabolism in a more complex way. When there is lack of nitrogen the growth rate is decreased. Under such conditions it was found that the yeast consumed ethanol regardless of a relatively high glucose concentration. (Woehrer and Roehr, 1981). Other nutrient limitations may also change the basic stoichiometric model (3.2). However, some of these limitations may be interpreted in terms of a decreased respiratory capacity (Käppeli and Sonnleitner, 1986).

3.4 Relation between uptake rates and concentrations

The substrate concentration has a primary influence on the uptake rate. For large concentrations the uptake rate saturates. Often a Monod type relation is used to express how the uptake rate depends on the substrate concentration over

a wide range under stationary conditions. However, under transient conditions the uptake rate may respond quite differently. A changed uptake rate results in changes in the overall activity of the cell and also in the cellular growth rate. It takes some time for the cell to adjust to such changes and reach a state of balanced growth that corresponds to the substrate uptake rate. One such estimate is about an hour (Dairaku *et al*, 1982). The uptake rate may depend not only on the substrate concentration but also on the status of the cell. This is not clear from the literature. Experimentally it is very difficult to measure the substrate concentration, since it is generally very low.

Rapid variations in the substrate concentration

Provided the fluctuations in the substrate concentrations are in the time scale of a few minutes, or shorter, the growth rate remains constant. In the subsequent study of fed-batch cultivation of yeast it is of interest to maintain cell growth at the critical rate where the yeast metabolism switches between ethanol production and consumption. For small variations in the substrate concentrations G and E, it is reasonable to assume a linear relation with the uptake rates, and combined with the bottle-neck principle the uptake rates would be

$$\begin{aligned} q_G(G) &= q_G^\circ + \alpha G \\ q_E(G,E) &= -q_E^\circ + \beta G, \quad E > 0 \end{aligned} \tag{3.14}$$

Note the sign convention on q_E. The bottle-neck principle implies that $\beta = \alpha\kappa$. For low glucose and ethanol concentrations, i. e when the full respiratory capacity is not utilized, it is reasonable to assume that the ethanol uptake rate is dependent on the ethanol concentration only

$$q_E(E) = \gamma E, \quad q_E < (q_{O_2}^{lim} - a q_G)/k \tag{3.15}$$

Adaptation of the yeast cell to a changed substrate concentration

The yeast cell adapts in several aspects to a sustained change in the substrate concentration. One important aspect for understanding the ethanol dynamics is that the respiratory capacity $q_{O_2}^{lim}$ would change (Rieger, 1983). The time constant is about an hour. This means that when the cells grow respiratively on glucose and then suddenly face a certain increase in the glucose concentration, they will start to produce ethanol and then grow respiro-fermentative for a time. However, the respiratory capacity increases slowly and eventually the metabolism becomes purely respirative again, at a higher rate. There is a definite upper limit of the respiratory capacity that is strain specific. When the yeast cells grow at the critical rate their respiratory capacity has reached its upper limit. Short time variations in the substrate concentration at the critical growth rate should not excite the dynamics of the respiratory capacity.

Another aspect of the substrate uptake rate is the internal storage of glucose in the form of trehalose and glycogen. This activity operates typically in the time scale of hours. However, yeast cells may for a short time rapidly accumulate glucose (Kätterer *et al*, 1985). This interaction with the internal glucose storage is significant at low growth rates but should not complicate the ethanol dynamics at the critical growth rate.

An asymmetry in the response of the cell to changes in the substrate concentration is apparent from the biology. The response to an increased substrate concentration is that a number of reactions are affected and speeded up, which eventually leads to an increased growth rate. A decrease in the substrate concentration, on the other hand, results in an immediate shortage of building material and lowered growth rate. The internal storage of glycogen and trehalose may even out some of these effects but this is not clear from the literature. The asymmetric response has been modeled mathematically (Lievense, 1984). For small variations of the substrate concentration in the short time scale such effects should not be significant.

4

Control of Fed-batch Cultivation

Feedback control of the substrate feed in baker's yeast production processes is slowly winning industrial application. Production is traditionally done in fed-batch using precalculated schemes for substrate pumping. Such schemes are the secrete behind good yeast making, and they are based on long industrial experience. Even if the environment conditions are held as constant as possible it is very difficult to get reproducible batches.

This chapter addresses feedback control of the substrate pumping from a biotechnical view-point. What is the result of feedback control in terms of improved yeast production? The subsequent two chapters deal with the actual design of the substrate control. Here a motivation for the work is given.

4.1 Industrial practice

Industrial production of baker's yeast has a long tradition. It is mainly produced batch wise. Continuous production is common at a laboratory scale but it is only recently evaluated for industrial production. Continuous operation gives a better utilization of the equipment compared to batch wise operation. However, problems with infections is a difficult technical problem. Further genetic drift of the culture is an inherent difficulty in continuous cultivation and this fact cannot be neglected in applications where the yeast quality is of major importance. Therefore production of yeast most certainly will be produced using fed-batch technique also in the future.

There is a lot of industrial experience in fed-batch cultivation of yeast. Much empirical knowledge is behind the formulation of dosage schemes. However, it is very difficult to obtain reproducible batches. The amount and quality of the yeast inoculum varies, and some manual adjustments of the feeding is quite normal. The yeast needs a certain feeding to grow optimally, while too much substrate starts ethanol production and causes the yield to go down. The yeast quality is also affected by the substrate dosage profile.

Commercial baker's yeast production involves several different steps and there are certain points that are more crucial for the economy and quality of the final product. The inocculum is cultivated in a series of increasing reactor sizes before it is entered to the production reactor. The media for the production stage, often molasses, is sterilized and carefully complemented to give a good growth yield. Apart from substrate dosage strategies the aeration of the reactor is a crucial factor. Much effort is done to design the reactors for good oxygen transfer. The control of pH is also important. Temperature control of the reactors is necessary. After cultivation the reactor broth goes through a separation step to remove water and unmetabolized products from the yeast. A short review of commercial yeast production is given in (Wallström, 1981). Even if there are several steps that influence quality and economy of the yeast production, cultivation conditions during the production stage remain a major factor.

4.2 Substrate control

To simplify, the culture is here considered as substrate limited. Sufficient aeration and nitrogen supply are assumed as well as good control of pH and temperature. Under this conditions the substrate control loop can be isolated from the other loops. Otherwise interactions may occur.

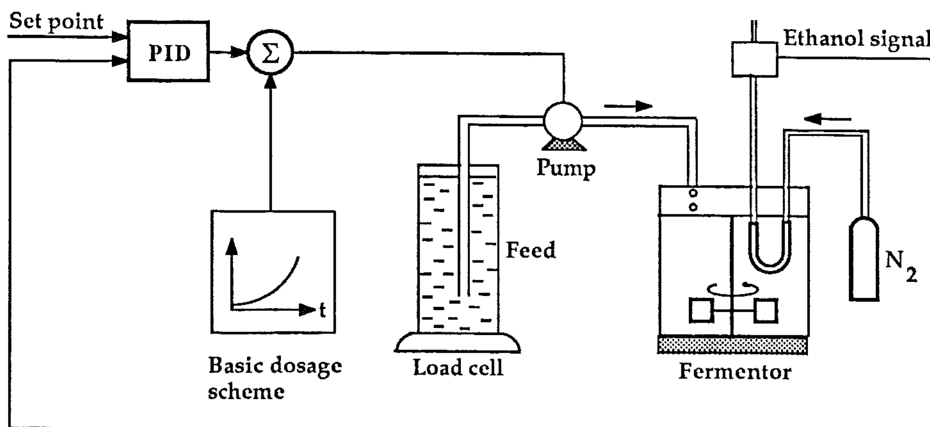


Figure 4.1 Experimental set-up for substrate dosage control.

There are different ways to monitor the actual substrate demand. Most of them are based on the fact that the cell metabolism switches at a certain substrate consumption rate and the substrate demand is considered to be this critical supply rate. There are also control principles based on estimates of the actual cell concentration and the assumption that the substrate demand is in proportion.

At over supply of sugar the cell culture start producing ethanol and when the sugar supply is too low, a combination of sugar and ethanol is consumed. Therefore ethanol measurement in the broth is a good indicator of the substrate demand. There are also other methods based on exhaust gas analysis. Changes in the respiratory quotient has been used as an indicator of the substrate demand.

In our laboratory on-line broth ethanol measurement was used for monitoring the substrate demand. The feed rate followed a precalculated basic dosage scheme and corrections from this scheme was made based on the ethanol signal. The experimental set-up is shown in Figure 4.1

4.3 Reproducibility of cultivations

Several cultivations were carried out using ethanol control and a certain degree of reproducibility was obtained. Here four cultivations are shown in detail, and experience from a number of other cultivations is summarized. Cultivations were grown from 3 g/L to 66 g/L in 18 h and the volume of the broth increased from 4.0 L to 6.0 L. The ethanol concentration was kept at a constant value. Results from the four cultivations are shown in Figure 4.2.

The set-point of ethanol was kept constant through many cultivations at a value of 0.4 g/L. This value was chosen somewhat arbitrarily. Our first cultivations were done with a set-point of $0.1 - 0.2$ g/L. During some of these cultivations a certain odour was present. This odour never occurred when the cultivations were grown with the high concentration set-point.

The four cultivations shown in Figure 4.2, were all carried out under almost identical procedures. The main difference was in the increase schemes for stirrer speed, resulting in different DO profiles. However, most of the time, the DO level was kept high, and variations should not have affected the cells. At certain moments, midway through cultivations B, C and D, there was an increase in the stirrer speed that led to an immediate increase in the DO level. These changes affected the feed rate/ethanol loop, and a pronounced increase in the feed rate was obtained.

Two overall characteristics of a cultivation are productivity and yield. Cell mass and the amount of consumed molasses at different times during the cultivations are shown in Table 4.1. The productivity was found to be remarkably reproducible, despite a variation in the DO level between the four cultivations. However, the yield varied substantially. It is not likely that the DO profile played

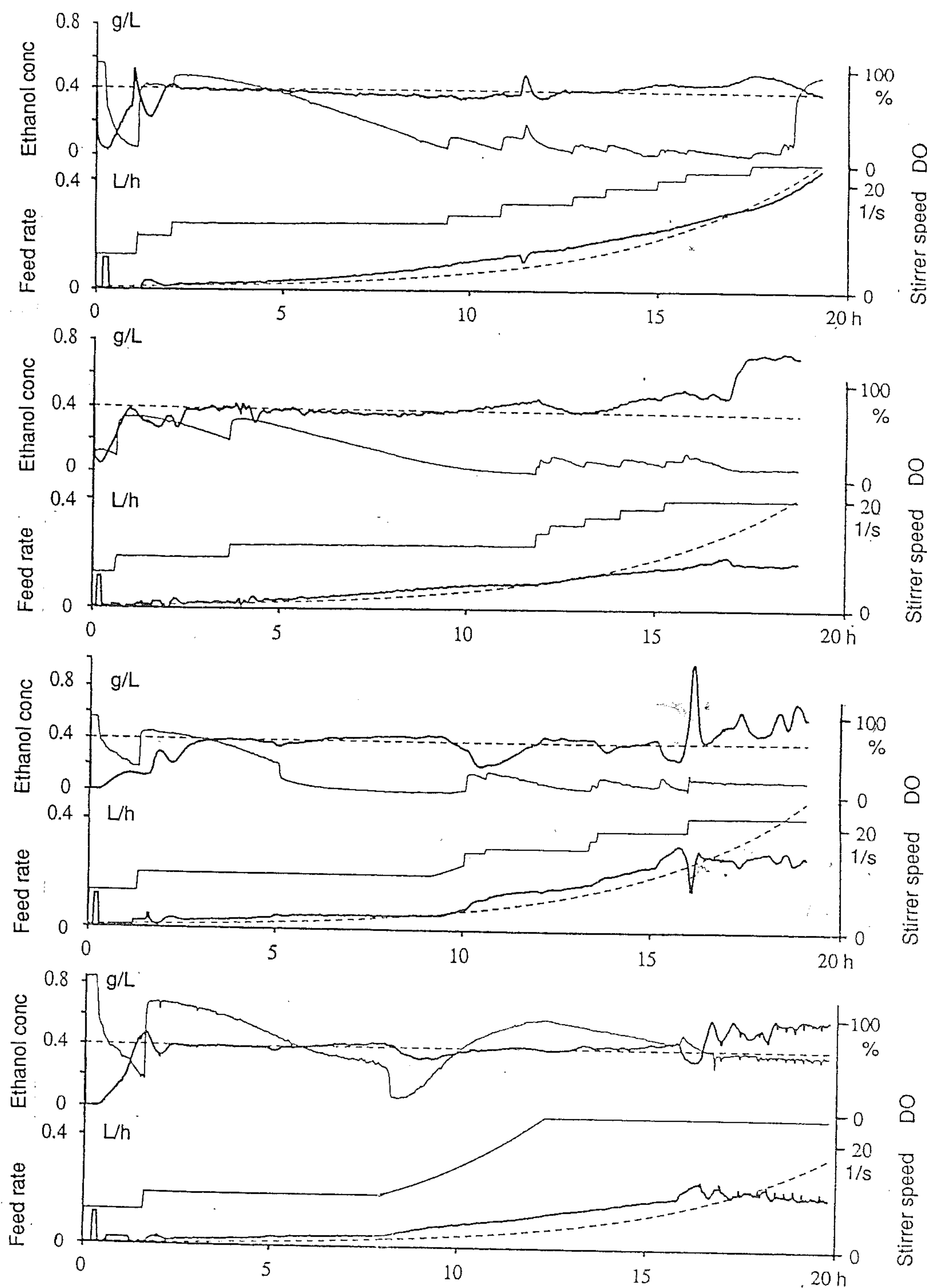


Figure 4.2 Registrations from four almost identical cultivations. The main difference was variations in the DO profile due to different schemes of stirrer speed. The regulator parameters were changed in cultivations C and D after 13.5 h. Lines: thick line = ethanol concentration and pump signal (upper, lower), dashed line = ethanol set-point and exponential basic dosage scheme, thin line = dissolved oxygen concentration and stirrer speed.

Table 4.1 Cell mass and consumed molasses at different times during the four cultivations A, B, C and D. The specific productivity μ_p, was calculated from the initial and the final cell mass. The yield Y, was calculated as g(yeast)/g(molasses). The molasses were from the same batch in all four cultivations.

Comment	*Time* [h]	x [g/L]	V [L]	*Feed* [g]	*Yacc.* [g/g]
A week : 8519	0.00	2.60	4.00	0	
$\mu_p = 0.20\ h^{-1}$	10.00	24.1	4.49	538	0.289
$Y = 0.25\ g/g$	12.00	33.7	4.75	855	0.279
	14.00	44.1	5.12	1270	0.270
	16.00	55.0	5.56	1794	0.262
	18.00	65.1	6.09	2407	0.256
B week : 8519	0.00	2.52	4.00	0	
$\mu_p = 0.20\ h^{-1}$	11.35	34.5	4.81	834	0.298
$Y = 0.25\ g/g$	13.30	42.3	5.08	1181	0.276
	15.30	50.7	5.49	1643	0.260
	18.30	65.3	6.03	2445	0.250
C week : 8547	0.00	3.50	4.00	0	
$\mu_p = 0.19\ h^{-1}$	11.05	24.5	4.66	578	0.276
$Y = 0.29\ g/g$	13.00	34.5	4.93	892	0.279
	16.00	54.2	5.64	1619	0.287
	17.30	64.8	5.97	2027	0.293
D week : 8547	0.00	2.58	4.00	0	
$\mu_p = 0.20\ h^{-1}$	10.00	21.4	4.49	452	0.302
$Y = 0.30\ g/g$	12.50	34.0	4.94	867	0.290
	15.50	51.6	5.57	1507	0.292
	17.50	66.8	6.00	2070	0.300

a role in this. There was actually a considerable difference in the DO profile even between cultivations with the same yield.

A closer investigation of the dry weight measurement taken during these cultivations, shows that up to a cell concentration of 35 g/L the variation in yield between the cultivations was well within measurement errors. The reproducibility was comparable with previous results on a medium of glucose, yeast extract, vitamins and minerals (Dairaku *et al*, 1981). Therefore conditions during growth from 35 to 65 g/L are believed to be the main reason for the 20 % variation in the yield between the cultivations, cf data in Table 4.1. When longer time periods are considered, the same measurement errors give rise to less uncertainity in the calculation of the yield.

From our data there are reasons to believe that factors other than variations in the ethanol concentration influence the yield. In the literature there have been some investigations concerning growth inhibitory substances. In a fed-batch process there is an accumulation of un-metabolized substances which might influence the growth of the culture (Maiorella *et al*, 1984). Another possibility

is the production of inhibitory substances during growth (Fukuda *et al*, 1978; Maiorella *et al*, 1983; Pons *et al*, 1986). In the recent work by Pons *et al*, there are indications for certain strains of *S. cerevisiae* that the level of ethanol concentration in the broth affects the production of an inhibitory substance, acetic acid. This is another indication of the importance of ethanol control.

4.4 Discussion

The objective of control is normally vaguely stated, high yield and growth rate, and a good and uniform quality. It is, however, laborious to evaluate the results of control in these terms. Previous work on ethanol control (Dairaku *et al*, 1981) was done using a medium of glucose, yeast extract, vitamins and minerals. In that work there is support for the hypothesis that constant ethanol control gives the optimal growth rate under the constraint of maximal yield. From our work with molasses, it may be concluded that ethanol control permits cultivation with high exponential growth up to a cell concentration of 60 – 70 g/L dry weight, maintaining a high yield. However, one may suspect that factors other than the metabolic state, are important for the yield. Such studies are still lacking also in the case of RQ control or growth rate control, for example.

An ethanol control system gives a tool for a new type of investigations of growth of yeast in a fed-batch reactor. There are indications in our work that the choice of ethanol set point influences the yeast culture. It would be interesting to study in more detail how yield, growth and quality depend on different ethanol set points and perhaps also to allow for different profiles of ethanol concentration during a cultivation. In the work (Pons *et al*, 1986) the level of ethanol concentration in the broth seems to affect the production of inhibiting acetic acid. Steady state experiments in a chemostat have shown that the ethanol production rate is related to the growth of a culture. Recently, a clear correspondence has been shown between ethanol production rate and growth rate also in a fed-batch reactor (Wu *et al*, 1985). There are indications that feeding strategies, which give different growth rates during various phases of the cultivation, might be important in obtaining good quality yeast (Dairaku *et al*, 1982; Shioya *et al*, 1985).

There are good reasons to believe that it is easier to evaluate experiments using ethanol control than using growth rate estimates based on optical density, exhaust gas analysis or pH. Each has its limitations. Direct measurement of the cell concentration using optical density is difficult. Methods based on exhaust gas analysis or pH require several model assumptions in order to interpret the signals. It is therefore less certain that cultivation conditions are reproduced using such feed rate control.

5

Process Model

A good understanding of process dynamics, disturbances and differences between batches, facilitates design of the control system. In Section 5.1 a process model is developed from mass balance equations. Under certain conditions ethanol dynamics can be simplified. A linear time-varying model of second order is derived. Identification experiments were performed to validate the model. These are discussed in Section 5.2. Results from several cultivations are shown and an idea is given of the parameter variation between batches. In Section 5.3 experience is mentioned from autonomous oscillations in the ethanol concentrations in the early experimental work. In Section 5.4 the character of disturbances and the degree of process uncertainty are discussed. The conclusions for control design are given in Section 5.5.

The results from the dynamical experiments are also interesting from a biotechnical point of view. They give insight into the dynamics of metabolic changes. This is important to know in order to understand effects of a large inhomogeneous reactor (Sweere *et al*, 1988).

5.1 Modelling

The yeast was grown in a well-mixed fed-batch reactor under substrate limited conditions near the critical growth rate where the yeast metabolism switches. The oxygen supply was assumed to be sufficient.

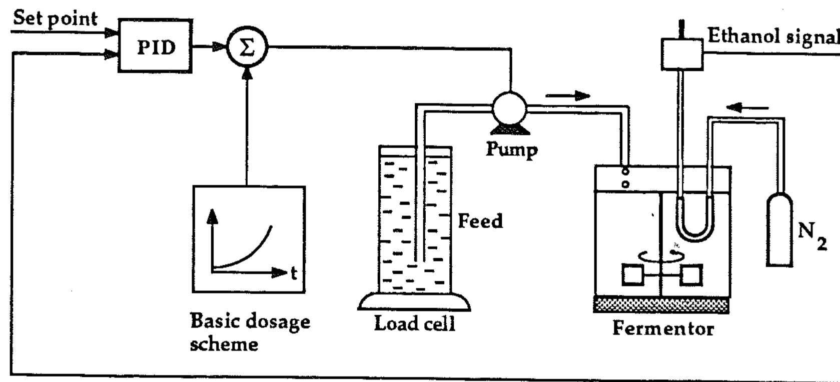


Figure 5.1 The experimental set-up.

Ethanol dynamics in a fed-batch reactor

Mass balance equations are given below. Note that the cell concentration X, as well as the volume of the broth V, increase during a cultivation. Let S denote the substrate and E the ethanol concentration in the broth.

$$\begin{aligned}
\frac{dV}{dt} &= F \\
\frac{d(VX)}{dt} &= \mu(S,E)\cdot VX \\
\frac{d(VS)}{dt} &= -q_S(S)\cdot VX + S_{in}\cdot F \\
\frac{d(VE)}{dt} &= q_E(S,E)\cdot VX
\end{aligned} \tag{5.1}$$

The relation between substrate uptake rates, cell growth and ethanol formation remains to be characterized. Here critical growth rate is assumed and only small variations in the substrate concentrations are considered. Further, the ethanol concentration is not allowed to reach inhibiting concentrations. The following approximations are then appropriate

$$\begin{aligned}
\mu(S,E) &= \mu_c \\
q_S(S) &= q_S^\circ + \alpha S \\
q_E(S,E) &= -q_E^\circ + \beta S, \;\; E > 0
\end{aligned} \tag{5.2}$$

Note that under these assumptions the differential equations become linear.

$$\frac{d}{dt}\begin{pmatrix} VS \\ VE \end{pmatrix} = \begin{pmatrix} -\alpha X & 0 \\ \beta X & 0 \end{pmatrix}\begin{pmatrix} VS \\ VE \end{pmatrix} + \begin{pmatrix} S_{in} \\ 0 \end{pmatrix} F - \begin{pmatrix} q_S^\circ \\ q_E^\circ \end{pmatrix} VX \tag{5.3}$$

The critical feed rate $F^0(t)$ to obtain stationarity in the ethanol concentration is

proportional to cell mass.

$$S_{in}F^{\circ} = (q_S^{\circ} + \frac{\alpha}{\beta}q_E^{\circ})VX \tag{5.4}$$

Metabolism operates in the time scale of a few minutes while cell growth is in hours. Over shorter time periods the ethanol dynamics can be approximated with a time invariant model.

$$T\frac{d\Delta S}{dt} + \Delta S = K_1 \Delta F \tag{5.5a}$$

$$\frac{d\Delta E}{dt} = K_2 \Delta S \tag{5.5b}$$

The parameters depend on volume and cell concentration as

$$T = \frac{1}{\alpha} \cdot \frac{1}{X} \tag{5.6a}$$

$$K = K_1 \cdot K_2 = \frac{S_{in}}{\alpha}\frac{1}{VX} \cdot \beta X = \frac{\beta}{\alpha} \cdot \frac{S_{in}}{V} \tag{5.6b}$$

The ethanol dynamics change during a cultivation according to (5.6) as cell concentration and volume increase. There are some factors in the reactor conditions that are not modeled in (5.2). For instance, the oxygen transfer is often a limiting factor at high cell concentrations. Further, unmetabolizable products of the feed and certain byproducts of cell metabolism, may accumulate and reduce growth.

Ethanol sensor and substrate feeding system

On a laboratory scale the measurement and the feed distribution become much easier than in a larger reactor. Since a homogeneous fermentor is assumed the placement of the sensor is not critical. The feed can also be assumed to be immediately mixed. Controllable high precision feed pumps are available at a reasonable cost. During a typical cultivation the flow increases from 0.01 L/h to 0.35 L/h.

Ethanol was monitored using a semiconducter gas sensor in combination with a membrane sampling probe immersed in the cultivation medium. The dynamics of the sensor system is dependent of the ethanol diffusion over the membrane, the transport to the gas sensor, and the subsequent adsorbtion on to the semiconducter surface. The dynamics is well described by

$$T_s\frac{dy}{dt} + y = E(t - T_d) \tag{5.7}$$

Typical parameters were $T_s = 2$ min and $T_d = 2$ min. The noise level of the sensor is low, however the non-linear characteristic may give severe problems in case of drift. It is further discussed in the section on modelling the disturbances.

The substrate feed rate was controlled by a peristaltic pump. Measures were taken to make the feed rate respond within seconds to a change in the pump signal. The feed tubing between substrate vessel, pump and fermentor was kept short, and any air-bubbles in the tubing were eliminated. The feed dropped into the broth from the top of the fermentor and it was easy to see that the drop frequency changed immediately when the pump signal changed. The actual feed rate was checked using data from the load cell.

Structure of the process transfer function

Insight into the control difficulties can be obtained by studying the process model in the frequency domain. A transfer function can be derived from the linear differential equations for the reactor (5.3) and the sensor (5.7). It has one fix and one time-varying part. The parameters K and T then depend on the volume and the cell concentration according to (5.6).

$$G(s) = G_{var}(s) \cdot G_{fix}(s) = \frac{K}{Ts+1} \cdot \frac{e^{-sT_d}}{s(T_s s+1)} \tag{5.8}$$

For control design it is important to consider the process transfer function around the bandwidth. A typical value lies in the range $5 - 20$ rad/h. At start-up there is a considerable phase lag. During the first few hours the phase increases for all frequencies. The gain variation is more complex. In a mid frequency band, around the typical bandwidth, the parameter variations in K and in T interact and make the gain first increase and then decrease.

5.2 Identification

Dynamical experiments were performed to validate the model. Special attention was paid to the dynamical properties of the ethanol sensor and the substrate feeding system.

Motivation for experiments in closed loop

The process model shows that it would be difficult to make reproducible dynamical experiments in open loop. The process contains an integrator and is thus not stable. The cell mass grows and the critical feed rate increases. Further, the process gain is high, and accentuates the difficulties to maintain constant ethanol concentration without feedback.

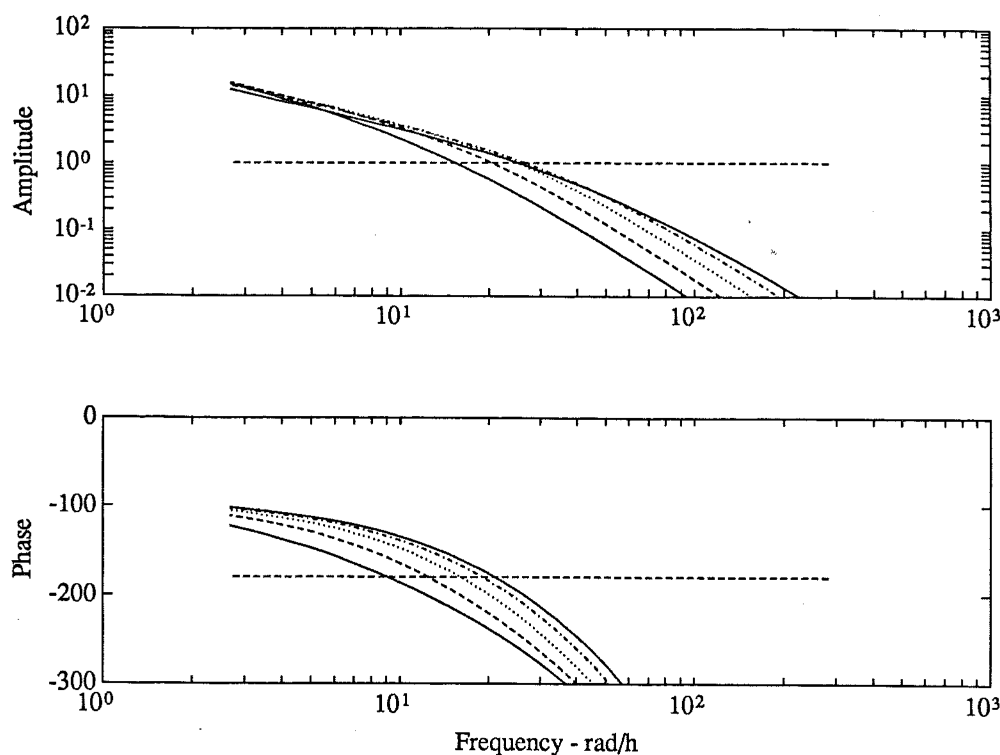


Figure 5.2 Bode diagram for the transfer function (5.8) at different times in a typical cultivation. Curves are shown for time 0, 4, 8, 12 and 16 hours of fed-batch cultivation. Parameters were $K = 50 - 70\ g/L^2$, $T = 0.5 - 10\ min$, $T_s = 2\ min$ and $T_d = 2\ min$.

Methods

The system was excited with a precalculated PRBS signal through the regulator ethanol set-point. The regulator was a PID regulator and the feed rate was controlled around an exponential basic dosage scheme, see Figure 5.1. The PRBS signal amplitude was chosen to $\pm 0.15\ g/L$ but at lower cell concentrations the amplitude was reduced to $\pm 0.05\ g/L$ in order not to saturate the feed rate signal. The basic period was 4 min in data from cultivation 1 and 2, and 6 min in data 3a, 3b and 3c. The sampling period was 0.5 min and the the control signal was kept constant between the sampling.

Statistical data analysis was done using the interactive program package MATLAB extended with the System Identification Toolbox (MathWorks, Inc). Maximum likelihood methods were used.

Inherent difficulties of the identification problem

There are several choices to make in order to obtain an informative experiment. Simulation studies are a good help.

In order to simplify the analysis of the data series, the length of the test

period is chosen to be no longer than that the variation in the parameters (5.6) has a negligible influence. On the other hand, a short test period sets a limit to the maximal time constant that can be distinguished from a pure integrator. Here a test period of about three hours was used, i. e. about 400 samples or 30 process time constants. In Figure 5.3 is shown a simulation where the time-varying model (5.5) is compared with the corresponding time-invariant model with averaged parameters.

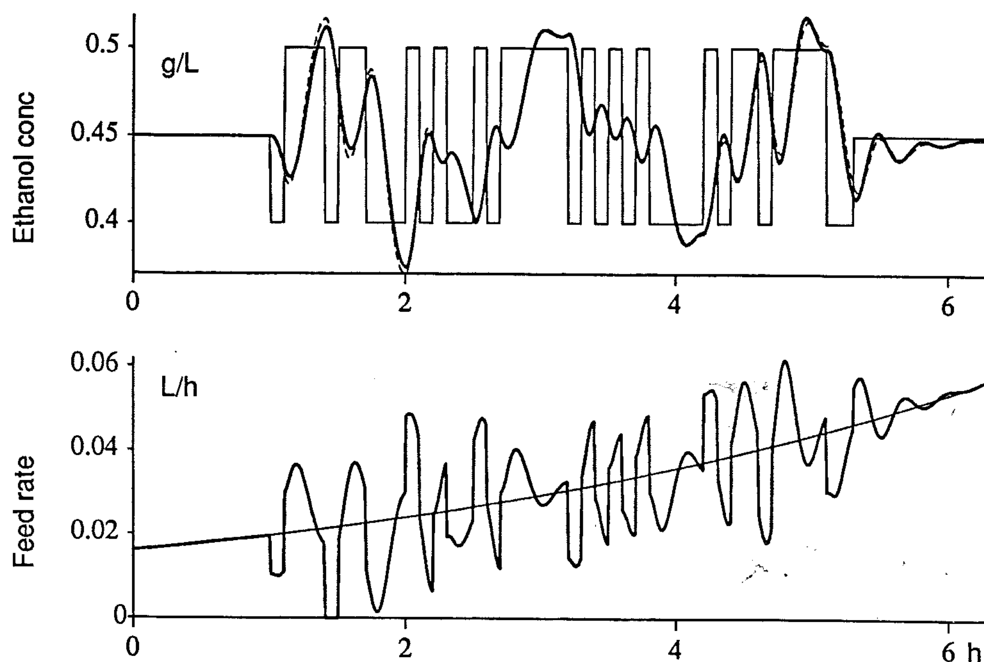


Figure 5.3 Comparison in simulation between a time-varying and a time-invariant model. Cell concentration varies between 5-10 g/L and volume from 4.0-4.2 L. Solid line is the time-varying model; Dashed line is the time-invariant model.

Minor differences between the dosage scheme and the critical feed rate could be reduced by the PID-regulator. However, larger differences may give rise to biases or trends in the measurement signal. Trends in both input and output signal are important to remove before process model identification.

The amplitude and frequency of excitation are crucial experiment parameters. It is important that the variation in the feed rate is no larger than that the model (5.5) is valid. This means that the feed rate should not be so low that the substrate vanishes and the ethanol consumption capacity saturates. Neither should the feed rate be too high so that the substrate concentration increases beyond the valid range of (5.2). The frequency of excitation was chosen so that the shortest peaks in the ethanol set-point should be noticeable in the ethanol signal.

The dynamics of ethanol production and consumption changes according to (5.6). The time constant T decreases typically from about 10 min at the start to 0.5 min at the end of a fed-batch cultivation. In this work the ethanol sensor had a time constant of about 2 min. This fact implies that it will be hard to determine T at high cell concentrations.

Dynamical properties of sensor and pump

Special attention was paid to the substrate feeding system and the ethanol sensor, both with respect to calibration and dynamical response.

The ethanol sensor was calibrated before and after each cultivation. The dynamics of the measurement system can be characterized by a transport delay time $T_d = 2$ min and $T_s = 2$ min. In Figure 5.4 the response of the sensor is compared with the result from such a model.

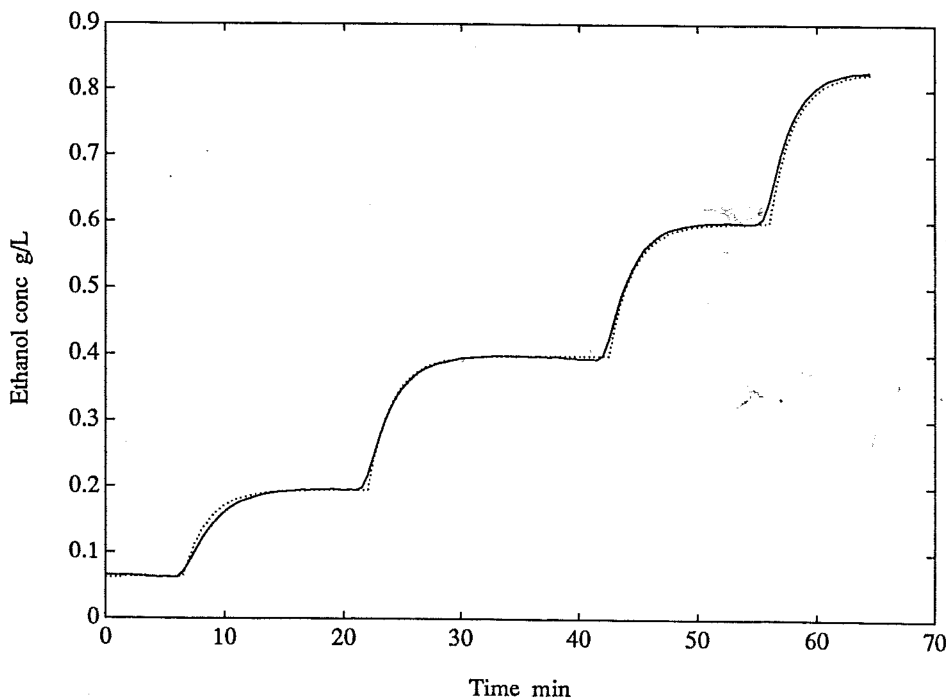


Figure 5.4 Dynamics of the ethanol sensor during calibration. The solid line is the measured ethanol signal and the dotted line is a first order model with the time constant $T_s = 2$ min. Data was from the calibration after cultivation 3.

Measures were taken to make the feed rate respond within seconds to a change in the pump signal. The feed tubing between substrate vessel, pump and fermentor was kept short, and any air-bubbles in the tubing were eliminated. The feed dropped into the broth from the top of the fermentor and it was easy to see that the drop frequency changed immediately when the pump signal changed. The actual feed rate was monitored by the substrate vessel load cell. In Figure

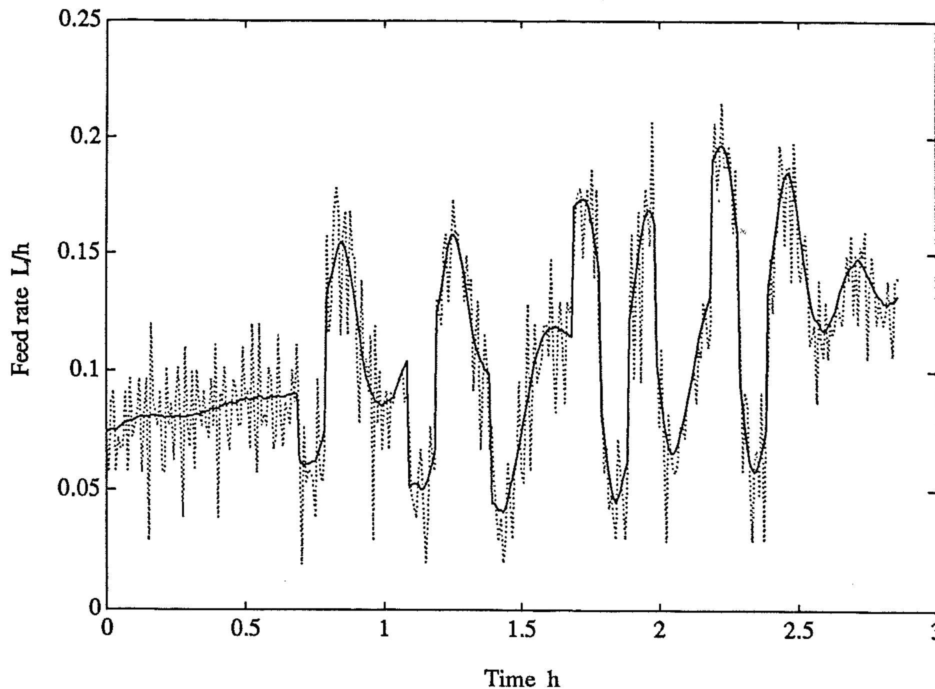


Figure 5.5 Comparison between the pump signal and the data from the load cell. The solid line is the pump signal and the dotted line is the differenced load cell signal. The data was taken from the start of test period 3b.

5.6 the pump signal is compared with the differenced load cell signal. The high noise level was mainly due to the quantization error of the digital load cell.

Results from experiments

Several identification experiments were done. In Figure 5.6. is shown raw data from three test periods during cultivation 3. The measured ethanol signal is compared with the output from the identified model in closed loop simulation. Note that the cell concentration increases considerable. See also Table 2. During the last test period the regulator was not able to keep the ethanol concentration down. The experiment was terminated before the PRBS sequence was finished. Note that the scales are changed between the test periods. To make comparison easy, the ratio between the ethanol and feed rate scales are kept constant.

Removing trends in the data

During some test periods the difference between the basic dosage scheme and the critical feed rate became substantial. The critical feed rate was estimated by fitting an exponential curve to the actual feed rate signal. However, in cultivation 3*c* a linear trend was more appropriate. By subtracting the estimated critical feed from the feed rate signal, the trend in the input signal was almost removed. Due

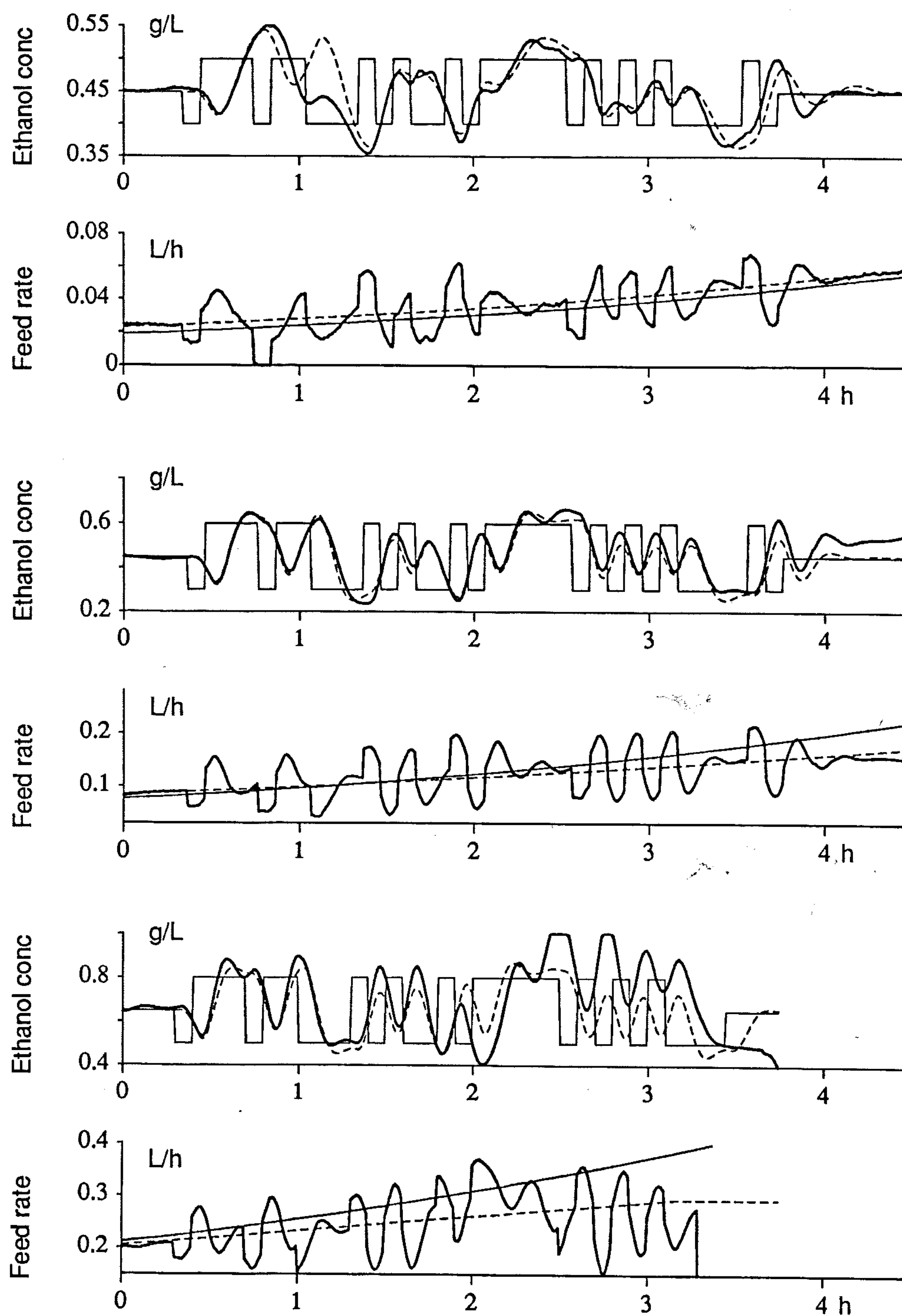


Figure 5.6 Three identification experiments: a, b and c, during cultivation 3. Thick lines are ethanol concentration and feed rate (upper, lower), thin lines are ethanol set-point and basic dosage scheme (upper, lower), and dashed line is the output from the identified model (upper) and estimated critical feed rate $\hat{F}^0(t)$ (lower).

to insufficient control a trend appeared also in the ethanol signal during some test periods. However, the trend in the measurement signal was close to a ramp and easy to remove.

The difficulties with removing trends in the feed rate signal are eliminated if identification is done between the set-point and the ethanol signal instead. However, it is difficult to interpret the closed-loop parameters in terms of process parameters.

Identified ARMAX models

The data was fitted to ARMAX models of different orders. The model order, delay and number of parameters were determined using Akaike test criterion and comparison of the models in the frequency domain (Ljung, 1987). Although a third order model is expected, only a second order model was motivated from the identification. The identified third order model gave the same frequency response as the second order model. The data fit was good and the residuals were white and not significantly correlated with the input. The obtained parameters showed the process integrator and one time constant.

Exploit the known integrator

In order to reduce the uncertainty in the estimate of process gain and time constant, the structure of the process integrator was included in the model by differencing the ethanol signal. Taking differences of the ethanol signal also reduces the effect of drifts due to poor control, on the identification. The data was fitted to a first order model, where $y(t) = E(t) - E(t-h)$, $u(t) = \Delta F(t)$, and $e(t)$ is a white noise residual. The sampling interval is denoted by h and the time delay by k.

$$\begin{aligned} y(t) = &- a_1 y(t-h) \\ &+ b_1 u(t-kh-h) + b_2 u(t-kh-2h) \\ &+ e(t) + c_1 e(t-h) \end{aligned} \tag{5.9}$$

Data from six experiments are summarized in Table 5.1. One example is shown in Figure 5.7, where data is from experiment 3b. Note the low noise level.

The data in Table 1. needs a comment. The identified pole a_1 can be interpreted as a combination of the process and sensor time constants. At higher cell concentrations the sensor time constant dominates. The variation in the estimated time delay k with dynamics, is due to the fact that the underlying process dynamics has higher order and introduces extra phase lag. This fast dynamics was not sufficiently excited and could not be identified more than as an extra time delay. The noise level is generally low but it changes character between the test periods. In 2a and 3a the C-parameter is large and it is believed to be due to the lower signal levels and correspondingly higher quantization error.

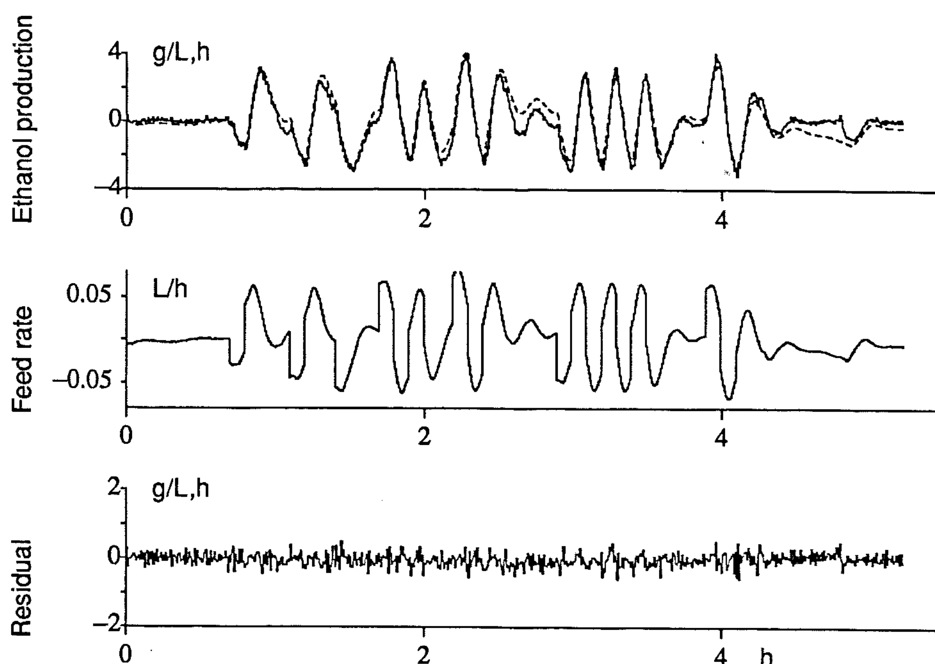


Figure 5.7 An example of differenced ethanol signal fitted to a first order model (5.9). The upper diagram shows the differenced ethanol signal divided by the sampling interval, solid line. The dashed line is the output from the model. The variation of the feed rate from the estimated critical feed rate $\hat{F}^0(t)$ is shown in the middle and the lower diagram shows the residual $e(t)$ after identification. Data is from experiment 3b.

Table 5.1 Identified parameters in a first order ARMAX model.

Data	k	a_1	b_1	b_2	c_1
1	5	−0.79	0.034	0.058	−0.27
2*a*	8	−0.94	0.044	−0.010	−0.83
2*b*	4	−0.93	0.027	0.025	−0.06
3*a*	7	−0.89	0.074	−0.018	−0.43
3*b*	4	−0.87	0.030	0.050	−0.04
3*c*	4	−0.84	0.031	0.067	−0.82

Explanation of identified time delay variation

There are good reasons to believe that the time-varying time delay is an artefact from the identification procedure. Identification on simulated data from the model (5.5)-(5.7) gives the same effect. The explanation is that the process is poorly excited and therefore only a second order model is identified though a third order model is expected. The remaining dynamics is approximated with

extra time delay. At increasing cell concentrations the neglected dynamics is faster and the extra time delay smaller.

The effect of a varying time delay can be understood in terms of model reduction. In Figure 5.8 results are shown from model reduction in the frequency domain (Lilja, 1988). The third order process model with time delay (5.8) is approximated with a second order model with time delay

$$\bar{G}(s) = \frac{\bar{K}e^{-s\bar{T}_d}}{(\bar{T}s+1)s} \tag{5.10}$$

In Figure 5.8 it is assumed that the parameters follows (5.6). The upper diagram shows the values for T, T_s and the approximation $\bar{T}$, for different cell concentrations. The lower diagram shows the corresponding values of the timedelay T_d and the timedelay of the approximate model, $\bar{T}_d$. The gains K and $\bar{K}$ are equal.

Identified process parameters

The continuous time parameters K and T in (5.5) can be calculated from the identified discrete time parameters in Table 5.1

$$K = \frac{1}{h}\frac{b_1+b_2}{1+a_1}, \quad T = -\frac{h}{\ln(-a_1)} \tag{5.11}$$

provided the sensor time constant is negligible compared to the ethanol dynamics. This relation is used to obtain the estimates $\hat{K}$ and $\hat{T}$ in Table 5.2. The identified time delays $\hat{T}_d$ are also given. The expected process gain $\check{K}$, from (5.6b) and the stoichiometry in Chapter 3 is given for comparison. Dry weight measurements of the cell concentrations were done before and after each experiment except for experiment 1, where the cell concentrations were estimated from the amount of feed consumed.

Table 5.2 Interpretation of the data in terms of process parameters.

Data	V [L]	X [g/L]	$\check{K}$ [g/L^2]	$\hat{K}$ [g/L^2]	$\hat{T}$ [min]	$\hat{T}_d$ [min]
1	4.2 – 4.6	10 – 21	40	58	2.2	2.5
2*a*	4.0 – 4.1	4.6 – 9.3	43	70	7.8	4.0
2*b*	4.8 – 5.4	30 – 48	35	88	6.6	2.0
3*a*	4.0 – 4.3	4.0 – 13	43	60	4.2	3.5
3*b*	4.4 – 5.2	19 – 40	37	72	3.5	2.0
3*c*	5.8 – 6.3	50 – 63	30	74	2.9	2.0

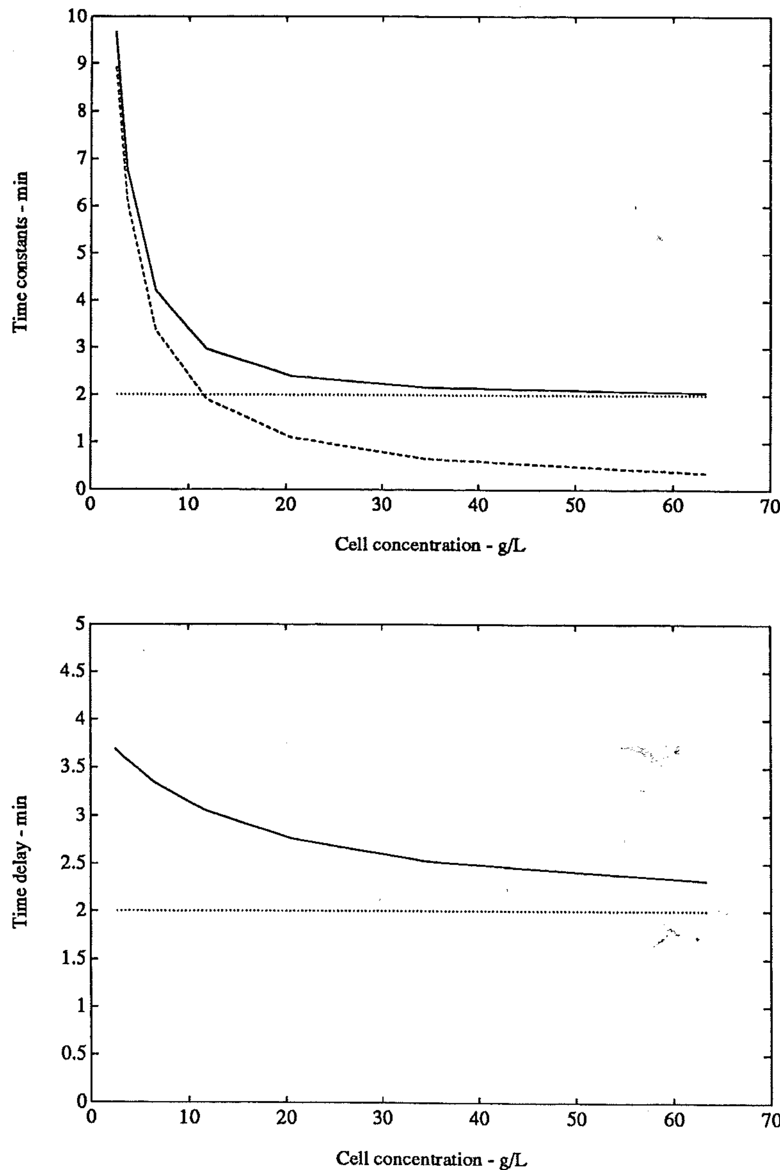


Figure 5.8 Approximation of a third order model with time delay to a second order model with a longer time delay.

Conclusions from identification

The results are in general very good. The ethanol dynamics show a deterministic behaviour and the noise level is very low.

A comparison between results from the same cultivation show an interesting result. The process time constant decreased towards the sensor time constant as expected. However, the process gain increased slightly while a slight decrease was expected from the model (5.6b). The identified process gain was found somewhat

higher than could be expected from the stoichiometric model.

A natural explanation for the higher process gain would be that the limiting oxygen transfer at high cell concentrations, also had an influence on the ethanol gain. However, the most immediate stoichiometric analysis does not reveal any relation between the availability of oxygen and the gain in the ethanol loop, as discussed in Chapter 3. Lack of oxygen would only decrease the critical feed rate.

5.3 Unexpected autonomous oscillations

The results from identification show a remarkable simple behaviour of the culture ethanol dynamics. This is somewhat misleading. The yeast sugar metabolism is a complex process and there is potential for a complex behaviour. Under certain conditions the cell culture may synchronize for instance, which give rise to autonomous oscillations in the concentrations of many substances in the reactor.

In the early work with yeast in our laboratory, strange variations in the ethanol concentration was recorded (Axelsson, 1985). The culture was grown under ethanol controlled conditions for several hours. Then autonomous oscillations started in the ethanol signal, with a period of about 10-20 min. A similar behaviour was found in the dissolved oxygen signal. The oscillations continued after the feedback loop was broken and the feed pump was set manually. An example of such autonomous oscillations is shown in Figure 5.9. These oscillations were treated as an hindrance of work. However, one observation is that in these early cultivations a semi-synthetic glucose media was used. Later, molasses was used and no such oscillations has been observed.

Observations of autonomous oscillations are sparsely reported in the literature. Early work (Meyenburg, 1969) that deals with effects related to synchronously growing cultures. The individual yeast cell produces and excret ethanol for about half an hour to an hour at the initiation of the budding phase. Usually, the cells in the fermentor are more or less uniformly distributed over the cell cycle, and the effect averages out. However, certain changes in the environment induce synchrony. Recent work (Sonnleitner *et al*, 1987) discusses mechanisms that enhances stable oscillations. This oscillations may give rise to difficulties in continuous cultures (Parulekar *et al*, 1986). There are also oscillations that are not correlated with the cell cycle. Interaction between the metabolic flow and reserve material in the cell, like glycogen and trehalose, may give rise to oscillations in the metabolism at growth rates below 0.15 h^{-1} (Heinzle *et al*, 1983).

Our records of autonomous oscillations are difficult to relate to the literature, because they have such a short period time. In general, stable oscillations in the culture seems to be poorly understood (Chance, 1973).

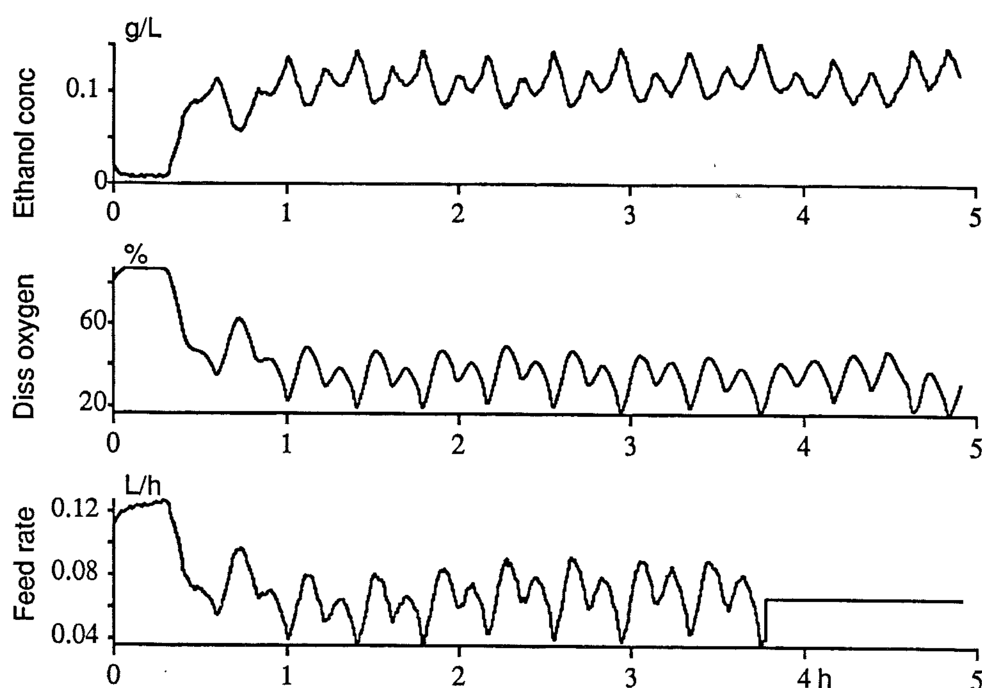


Figure 5.9 An example of stable autonomous oscillations in the ethanol signal.

5.4 Disturbances and process uncertainty

It is important to consider likely disturbances in the design of a control system. Here is discussed variations in the fermentation as well as disturbances introduced through the pump and the sensor. There is also differences in the process from cultivation to cultivation. Especially the critical feed rate profile differs. The disturbances could be divided into two time scales, and one should distinguish between differences between cultivations and variation during a typical cultivation. The discussion in this section leads up to a background for evaluating the robustness of a control system.

Variations in the exponential feed demand

The most apparent disturbance during the cultivations was the deviation in the feed demand from the exponential dosage scheme. The decrease in the feed rate growth was related to a decrease in the cell growth rate at high cell concentrations. The oxygen transfer might be a limiting factor, and certain products may accumulate and reduce the growth rate as well. Typical examples can be seen in the data series shown in Figure 4.2.

The feed rate profile also differs considerably between cultivations. The size and quality of the inoculum vary. These differences have a drastic influence on the feed demand during the latter half of the cultivation.

Sensitivity to errors in the feeding system

An observation from the process model is that the relative sensitivity to variations in the feed rate increases considerable during a fed-batch cultivation. It increases with cell density, since the critical feed rate grows as cell mass (5.4), and the process gain K remains almost constant, (5.6b) and Table 1.

This means that the pump accuracy should be measured in absolute terms. Here an high precision peristaltic pump was used. There was no problem to maintain good precision throughout a cultivation. The actual feed rate was checked afterwards using the load cell data. The pump signal was compared with differences in the weight measurement signal, and the pump gain and the pump bias were estimated. Our experience is that the bias was close to zero while the gain usually varied about 10% between cultivations.

On an industrial scale it is common to use several pumps to achieve accuracy over a wide range of feed rates. A typical installation could use combinations of pumps with different capacities which are turned on and off according to the feed rate demand. Then, on top of that, a special pump with variable feed rate could be used for control purpose. The on-off control of the larger pumps is likely to influence the ethanol control loop. Step disturbances at the feed rate is a reasonable model to account for this difficulty, when a control system is designed.

Deterioration of the ethanol sensor

The ethanol sensor was calibrated before and after each cultivation. The fact that the sensor has a pronounced non-linear characteristic makes it sensitive to parameter variations. An observed bias in the calibration may also result in a gain variation.

The dynamics of the sensor did not change. Only extraordinary circumstances made the time constant and the time delay increase. However, fouling of the membrane was never a problem. The noise level of the sensor was negligible.

Gain variations due to reactor phenomena

There are several factors that may influence the process gain. Drifts in the sensor and pump calibrations are already mentioned. The substrate concentration of the feed is another source of uncertainty. Molasses is a complex substrate and contains many differents sugars. The industrial experience is that concentration of fermentable sugars may vary with 15 %. In the identification experiments all these uncertainities were eliminated. Despite these precautions there is a deviation of the identified process gain from what was expected. The process model implies that the gain should decrease as the volume increases (5.6b). However, from the identification studies the tendency was that the gain increased slightly during the cultivation.

In the ethanol controlled cultivations in Figure 4.2 there are several several examples of how a low dissolved oxygen concentration has decreased the feed rate

increase. When the dissolved oxygen concentration increased due to change of stirrer speed, the feed rate increased. These examples did not show any tendency to unstable control actions. There is no evidence so far of an increased gain in the ethanol loop due to low dissolved oxygen concentrations.

Under extraordinary conditions there are of course many factors that might influence the process gain in the substrate control loop. During one cultivation for example, low *pH* caused unstable control actions (Axelsson, 1987). Such disturbances should be detected and cause an alarm, rather than be compensated for by adapting the regulator.

Differences between cultivations

The accumulated experience over a two year period with ethanol controlled yeast cultivations is that the process differs considerable in some respects, (Axelsson, 1987) and (Axelsson *et al*, 1988). The differences in the feed rate profile is striking, see Figure 5.10. This difference in the feed rate demand from cultivation to cultivation, shows that feed-back is valuable to maintain a precise respiratory state of the yeast culture throughout a cultivation.

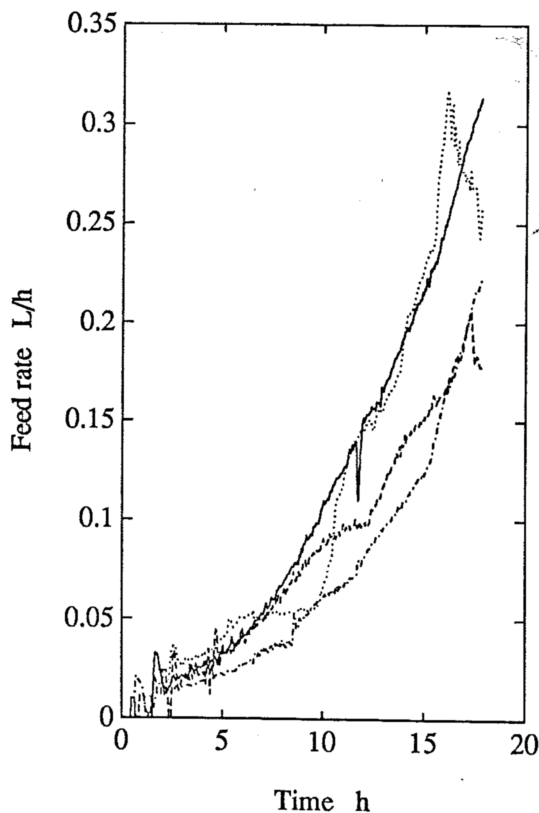


Figure 5.10 Feed profiles in six cultivations when the broth ethanol concentration was kept constant.

The identification results from different cultivations show some variations in the process gain. Part of these differences were due to drift in the calibration of the pump and this have been compensated for before presenting the data. There were also differences in the identified process time constant.

5.5 Discussion

The main reason for control is to track the exponential increase in the feed rate demand. The uncertainty in growth rate and in initial cell mass makes it of limited value to use only a precalculated dosage scheme. It is hard to avoid an exponentially growing load.

The time delay and dynamics of the sensor set a constraint on the magnitude of the control gain that can be used with maintained stability. In other words, there is a relation between the response time of the sensor and the deviation in the exponential feed demand that can be compensated.

The limitation imposed by the sensor delay on the control system, can be diminished if the process is predictable in the time scale of the sensor response. A good process model is therefore of great interest. Part of the dynamics is well-known. The time-varying, more uncertain part can be described in terms of a first order system. The structure of the uncertainty may be exploited in control design.

The results from the identification experiments promise good possibilities for short term prediction. If on-line estimation of the process model were to be implemented, the level of excitation could only justify a reduced order model. However, a reduced order model means that the estimated time delay varies considerably and it should be accounted for in the estimation algorithm. The change in the process time constant is quite predictable and could be exploited in the algorithm. A quantitative robustness analysis of the closed loop performance is called for, in order to determine how accurate the short term prediction ought to be. The control system must also be designed to manage reasonable differences in the equipment gains between cultivations. An estimate of the process gain with an uncertainty of a factor two should be safe.

The demands on the control change character during the cultivation. The exponential load on the system is stressed here. It becomes pronounced mainly during the latter half of the cultivation. Further, on an industrial scale, step disturbances in the feed rate may be more frequent during this period. At low cell concentrations, during the start-up of the cultivation, the feed rate growth is small. The main difficulty is then the slow dynamics in the reactor combined with the fact that the control authority is low. It is important that an overshoot in the ethanol concentration is avoided, because of the long time required for the cells to consume it. The different character of the control difficulties at start-up and late in the cultivation may call for different regulators.

6

Design of the Ethanol Control Loop

Major aspects of the substrate control design for fed-batch cultivation of yeast is discussed in this chapter. The control is based on on-line measurement of ethanol in the broth and part of the process knowledge obtained in the previous chapter is used. The main reason for control is to track the drastic growth in substrate demand.

First the control problem is characterized in Section 6.1. In Section 6.2 the possibilities with PID control is analysed. Section 6.3 discusses tuning. In Section 6.4 an observer is designed for the substrate demand and the robustness of the closed loop system is analysed. A short discussion in Section 6.5 concludes the chapter.

6.1 Characterization of the control problem

In the previous chapter a process model was developed and the control difficulties were described. It is here shortly recapitulated to give a basis for control design. The experimental set-up is shown in Figure 6.1.

Process model

In the previous chapter it was discussed how the cell density X and the volume V slowly increase. Actually it takes about 3-4 hours for the cell density to double. The ethanol concentration on the other hand reacts to changes in the substrate feed rate in the time scale of a couple of minutes.

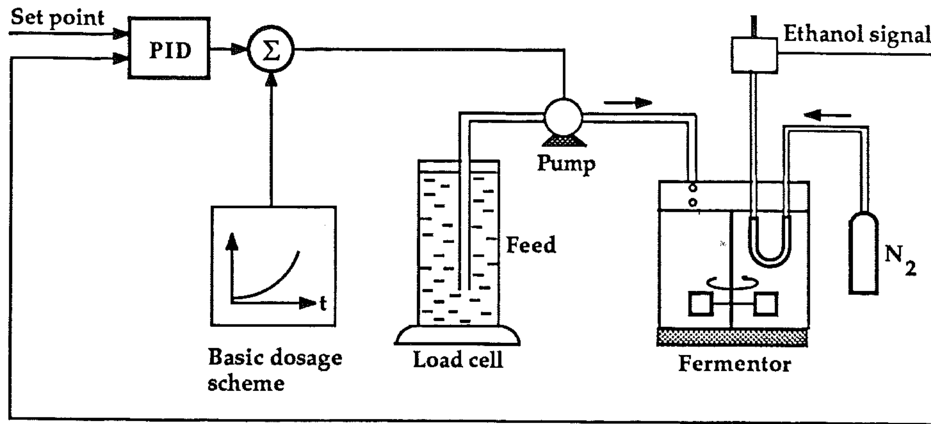


Figure 6.1 The experimental set-up.

The feed rate demand $F^{\circ}(t)$ is proportional to the cell mass, see (5.4). It can be approximated with an exponential function

$$F^{\circ}(t) = (F_0 + \delta F_0)e^{(\mu+\delta\mu)t} \tag{6.1}$$

where the parameter deviations δF_0 and $\delta\mu$ represents process uncertainty.

To describe the ethanol dynamics around the critical feed rate $F^{\circ}(t)$ it was found useful to introduce a second order linear time-varying model containing a pure integrator. Cell density X and volume V enter the model through the parameter variations of the gain K and the time constant T. The broth ethanol measurement system is well described by a time delay T_d and a time constant T_s. The total process transfer function G_p is given below. It describes the dynamics for small variations u in the feed rate F around the basic dosage scheme F_b, to deviations in the ethanol concentration y around a low value. Note that it can be structured into one fix and one time-varying part.

$$G_p(s) = G_{var}(s) \cdot G_{fix}(s) = \frac{K}{Ts+1} \cdot \frac{e^{-sT_d}}{s(T_s s+1)} \tag{6.2}$$

where the parameters vary as

$$K \sim \frac{1}{V}, \quad T \sim \frac{1}{X}, \quad F^{\circ} \sim VX \tag{6.3}$$

There are some factors in the reactor conditions that are not modeled in (6.3). Results from identification experiments showed actually that the process gain K increased slightly with time rather than decreased. One factor that is not modeled is that the oxygen transfer may become a limiting factor at high cell concentrations. Further, unmetabolizable products of the feed and certain byproducts of

the cell metabolism, may accumulate and reduce growth. Such changed conditions would also influence the process dynamics. The most immediate stoichiometric analysis given in Chapter 3.3, shows, however, that the gain would remain constant despite a decreased oxygen supply.

Main control objective

The main reason for control is to track the exponential increase in the feed rate demand $F^{\circ}(t)$. It can be considered as a load disturbance at the process input. In the control system it is natural to incorporate knowledge of the growth characteristic of the culture, for instance in terms of a basic dosage scheme $F_b(t)$. The difference between the critical feed rate $F^{\circ}(t)$ and the basic dosage scheme $F_b(t)$ remains as a load disturbance. The control system is shown in Figure 6.2.

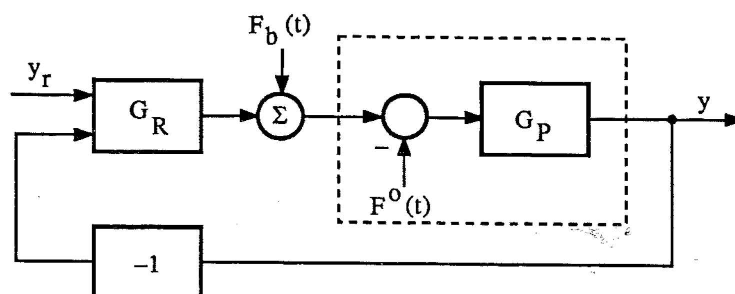


Figure 6.2 Block diagram of the substrate control system.

The time delay and dynamics of the sensor set a constraint on the magnitude of the control gain that can be used with maintained stability. The fact that the process dynamics change as the cell culture grows actually poses only minor difficulties. At start-up there is a considerable phase lag. During the first few hours the phase increases for all frequencies. The gain variation of the model is more complicated. In a mid frequency band, around a typical bandwidth, the parameter variations in K and in T interact and make the gain first increase and then decrease.

In Figure 6.3 is shown the performance of PI control around a basic dosage scheme. Most striking is the drift to a higher ethanol concentration during the latter part of the cultivation. The deviation is due to a miss-match between the basic dosage scheme and the actual substrate demand. Further, the performance of the feedback varies during a cultivation. At start-up the loop gain is high, and the regulator is almost oscillating. Later the regulator is not strong enough to keep the ethanol concentration down. This is an effect of the variation in the dynamics of the process as discussed above.

The fed-batch process is finished after a finite time and that fact has consequences for control design. The exponentially growing disturbance is a difficult

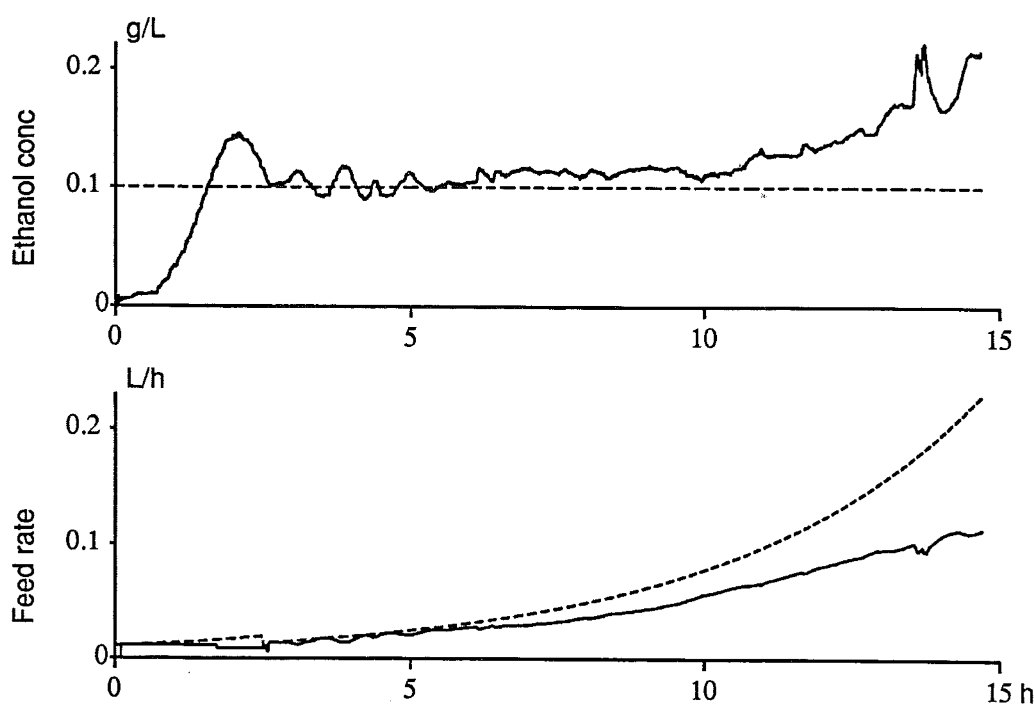


Figure 6.3 A typical example of PI-control. In the lower diagram is shown the feed rate $F(t)$, solid line, and the basic dosage scheme $F_b(t)$, dashed line. The basic dosage scheme was manually adjusted after 2.5 h of cultivation.

problem but it must be considered only over a given time interval. Discussions about asymptotic behaviour are not relevant. Another consequence is that there is a limited time for tuning of the regulator to the actual process. Automatic tuning procedures are only interesting if they are rapid.

6.2 Possibilities with PID control

It is natural to first explore the possibilities with conventional PID control. Tuning of the PID regulator and understanding of its limitations can give valuable insight into the control problem and the role of adaptivity. Modification of the regulator structure is also discussed.

In fact, PID control have been used for dozens of cultivations with a reasonable performance (Axelsson *et al*, 1988). Different ways to change the regulator parameters during the cultivation have been discussed in the literature (Dairaku *et al*, 1983) and (Axelsson *et al*, 1984). Here, the characteristics of the disturbance is emphasized and the role of the varying process dynamics is played down.

Integral action and basic dosage scheme

To study what disturbance rejection that is possible to obtain, the process is first approximated as an integrator and the derivative part of the PID-regulator is neglected. In the next section it is then discussed how the stability is augmented using the D-part and the more detailed model (6.2). The low frequency behaviour is thus described by the approximate transfer functions

$$G_p(s) = \frac{K}{s} \tag{6.4a}$$

$$G_{PI}(s) = K_R(1 + \frac{1}{T_I s}) \tag{6.4b}$$

giving the closed loop transfer from the load disturbance d to the output y as

$$G_d(s) = \frac{Ks}{s^2 + KK_R s + KK_R/T_I} \tag{6.5}$$

The critical feed demand can be approximated by an exponential function (6.1), and $\mu \approx 0.2\ h^{-1}$ and $F_0 \approx 0.01\ L/h$ are typical parameters. Closed loop time constants in the order of 10 min are achievable, so initial transients die out within an hour. The response to this load disturbance would thus be

$$y \approx -G_d(\mu) \cdot F_0 e^{\mu t} \approx -\frac{T_I \mu}{K_R} \cdot F_0 e^{\mu t} \tag{6.6}$$

With reasonable parameters, $T_I/K_R \approx 3$, the deviation $|y(t)|$ may grow from 0.004 g/L to about 0.10 g/L in about 15 hours. Despite the integral part in the controller it is impossible to keep the desired ethanol concentration.

A substantial improvement is obtained if an approximation of the feed demand F° (6.1) is included in the regulator as a basic dosage scheme

$$F_b(t) = F_0 e^{\mu t} \tag{6.7}$$

Now the ethanol response would be

$$\begin{aligned} y(t) &\approx G_d(\mu) F_b(t) - G_d(\mu + \delta\mu) F^\circ(t) \\ &\approx \frac{T_I}{K_R} \cdot \mu F_0 \left(e^{\mu t} - (1 + \frac{\delta\mu}{\mu})(1 + \frac{\delta F_0}{F_0}) e^{(\mu + \delta\mu)t}\right) \end{aligned} \tag{6.8}$$

which for good estimates means almost total elimination of the ethanol error. On the other hand it could be worse if the estimates were off by a factor of two. Actually if $(\mu + \delta\mu)$ were as little as 25 % larger than μ the term $e^{(\mu+\delta\mu)t}$ would be twice as large as $e^{\mu t}$ after 15 hours, giving an $|y|$ of the same size as without the basic dosage scheme. If $F_b(t)$ is kept smaller than the demand $F^\circ(t)$ it follows that $|y|$ is always smaller than for $F_b(t) = 0$ but it leads to a

decreased ethanol concentration. A too low ethanol concentrations may result in bad growth conditions, and an overestimated basic dosage scheme is the therefore often prefered.

It should also be mentioned that feedback control is really needed to keep small ethanol deviations even if $F_b(t)$ were a good approximation of the demand. Any deviation between F_b and F° would be integrated, and in the later part of a cultivation substantial ethanol errors would result within a couple of minutes.

Derivative part

The closed loop system (5) with $KK_R/T_I \approx 25\ h^{-2}$ and $KK_R \approx 8\ h^{-1}$ would have a damping of $\zeta = 0.8$, which indicates a well damped system. However, if you include the neglected high frequency dynamics of (6.2), the phase margin drops from about 70° to as little as 5° for $T \approx 6\ min$. The stability has to be improved. A moderate derivative part with derivative time 6 *min* and high frequency gain $N = 4$ adds phase advance at the crossover frequency giving a phase margin of about 40°. The resulting PID-regulator preserves stability also for a process time constant of about 15 min, a doubled time delay, or a factor two in process gain. No excessive noise magnification is introduced.

It should be noted that although the regulator is fairly robust, this parameter setting requires quite a bit of knowledge of the process and some skill in the use of a Bode diagram. A computational tool like PC-Matlab is certainly of great advantage. Similarly it would require a lot of experience to tune the regulator at the process.

6.3 Adaptation of PID-regulators, alternatives

The tuning of the PID-regulators was found to be quite tricky. The low frequency requirements are strict and the process variation makes it difficult to match the phase advance to the crossover frequency. As discussed in the previous chapter, the process variation is partly known. The process time constant T in (6.2) decreases by a factor of about 25 in a well-known way during a batch. Parameter scheduling of especially the derivation time T_D of the regulator would enhance the stability robustness. Another parameter to adjust would be the integral time T_I. At the start of the batch there is very little disturbance load and the strong integral action of the regulator is really not called for. A much larger T_I, like 1-2 h, would be sufficient and greatly enhance the stability robustness. A similar reduction would certainly be necessary in order to maintain stability, if the sensor were slower.

The regulator design makes use of a lot of process knowledge. It would have been nice to go to the process with an auto-tuning regulator, and let it adjust to the specific batch. This is not so easy. The length of the batch is limited and the process is quite slow, at least in the beginning. A simulation of a relay auto-tuner

(Åström and Hägglund, 1984) shows that about two hours are required for three periods, and this is much to long. It does not give enough information either for the fine tuning of the derivative part.

One could decrease the relay period by introducing phase advance in the loop and that would give better estimates of the behaviour at the desired crossover frequency. However, it still requires about an hour of relay experiments. Another complication is the load disturbance. The simulations were done around a correct basic dosage scheme, and although the initial feed estimates are quite good it is known (Hang and Åström, 1987) that a changing load disturbance might influence the estimate of the process dynamics.

What is promising is to close the loop including a nominal PID-regulator around a low amplitude relay and then adjust especially the D-part according to the resulting relay-period. How this should be done and what performance that could be achieved is so far an open question. It requires much work both using simulation and analysis and experiments at the real process. In fact relay oscillations might excite more dynamics of the cell culture. The cells have different metabolism in different phases of cell cycle, and if oscillations in the substrate flow cause the cell population to synchronize, it may induce sustained oscillations (Parulekar *et al*, 1986). Such a behaviour was actually found in early cultivations using glucose as substrate instead of molasses, see Section 5.3.

The main reason for feedback control is the exponentially growing feed demand and the variation in this demand. A well-known principle is, that regulators should contain an internal model of the disturbance they are supposed to eliminate (Bengtsson, 1977; Åström and Wittenmark, 1984). The I-part of the PID-regulator could be viewed as a model for a constant load disturbance. It is intuitively reasonable to try to include a model of the exponential load-increase in the regulator. This is done in the next section.

6.4 Observer for the exponential feed demand

In the previous section it was found that a regulator with integral action was not sufficient to reject the exponential load disturbance. A regulator with an internal model of the disturbance has a potential to eliminate its influence. Actually one could regard the basic dosage as a model of the load. If that model is supplemented with feedback, i. e. an observer is used, the requirement of good estimates of the initial feed demand could be relaxed.

Derivation of a reduced order observer

The feed demand, $F^\circ(t) = F_0 e^{\mu t}$, grows slowly, and a simple observer can be based on $G_p(s) = K/s$ as the process model. Introduce the state variables $x_1 = y$ and $x_2 = F^\circ$ and μ as an estimate of the true growth rate $\mu + \delta\mu$. The observer

and the corresponding simplified model are then

$$\begin{cases} \dot{x}_1 = -Kx_2 + Ku \\ \dot{x}_2 = (\mu + \delta\mu)x_2 \end{cases}$$
$$y = x_1 \tag{6.9}$$
$$\begin{cases} \dot{\hat{x}}_1 = -K\hat{x}_2 + Ku + K_1(y - \hat{x}_1) \\ \dot{\hat{x}}_2 = \mu\hat{x}_2 - K_2(y - \hat{x}_1) \end{cases}$$

where K_1 and K_2 are the observer gains. The ethanol measurement signal has a low noise level, and it is natural to use a reduced order Luenberger observer instead of the full state observer (Kailath, 1980). The estimate $\hat{x}_2$ of the feed demand could then be obtained as

$$\frac{d\hat{x}_2}{dt} = \mu\hat{x}_2 - K_O\left(\frac{dy}{dt} - K(u - \hat{x}_2)\right) \tag{6.10}$$

A Luenberger observer is implemented without any measurement signal derivatives by using a direct feed through from y to $\hat{x}_2$ as seen from the Laplace transformation of (6.10). Let $\hat{x}_{20}$ denote the initial state of the observer.

$$\begin{aligned} L\{\hat{x}_2\} = & \frac{1}{s - \mu + KK_O}\hat{x}_{20} \\ & - \frac{k}{s - \mu + KK_O}(sL\{y\} - KL\{u\}). \end{aligned} \tag{6.11}$$

A state feedback

$$u = -K_R y + \hat{x}_2 \tag{6.12}$$

would then give perfect disturbance elimination, when $\hat{x}_2$ is equal to the feed demand x_2.

The two input form (6.11) and (6.12) of the regulator is valuable for the design of features for anti-windup and manual/automatic mode changes. Neglecting such nonlinear effects the internal regulator feedback would give the observer based compensator as

$$L\{u\} = -\left(K_R + \frac{K_O(s + KK_R)}{s - \mu}\right)L\{y\} + \frac{1}{s - \mu}\hat{x}_{20} \tag{6.13}$$

The regulator thus consists of three parts, a proportional feedback gain, an unstable dynamical part, and one part that could be interpreted as an exponential basic dosage scheme.

Load disturbance rejection

Similarly to the analysis of the PI-regulator it follows from the process model (6.4a) and the regulator (6.13) that the low frequency transfer from a load disturbance d to the output y could be described by

$$G_d(s) = \frac{K(s-\mu)}{s^2 + (KK_R + KK_O - \mu)s + KK_R(KK_O - \mu)} \tag{6.14}$$

The disturbance d is the difference between the approximation $\hat{x}_{20}e^{\mu t} = F_0 e^{\mu t} = F_b(t)$ of the feed demand and the actual demand $F^\circ(t) = (F_0 + \delta F_0)e^{(\mu+\delta\mu)t}$.

A closed loop bandwidth of about 10 min is achievable, as discussed below, so the initial transients die out within an hour giving the remaining ethanol response as

$$\begin{aligned} y &= G_d(\mu)F_b(t) - G_d(\mu+\delta\mu)F^\circ(t) \\ &\approx -\frac{\delta\mu}{K_R(KK_O-\mu)}F^\circ(t) \end{aligned} \tag{6.15}$$

Comparison to the corresponding expression (6.8) for the PI-controller it is seen that errors δF_0 in the estimate of the initial feed demand $F_0 = \hat{x}_{20}$ are totally eliminated, while the improvement achieved for errors $\delta\mu$ in the growth rate μ is less obvious. The regulator (6.13) closely resembles the PI-regulator (6.4b) with $K_R^{(PI)} = (K_R + K_O)$ and $T_I \approx 1/KK_R + 1/KK_O$. Comparable parameters give

$$\frac{y_{PI}}{y_{obs}} = 1 + t\mu \cdot \frac{1 - e^{-t\delta\mu}}{t\delta\mu} > 1 \tag{6.16}$$

Typical values are $\mu = 0.20\ h^{-1}$ and $\delta\mu = -0.05\ h^{-1}$ and this gives after 15 hours a factor 5 in stronger load rejection for the observer based regulator.

Stability margins and phase advance

The substantial improvement in load rejection (6.16) was obtained with comparable parameters and it is reasonable to slightly reduce the "integral action". Just as in the PID-case some phase advance is required for "integration times" T_I as short as 20 min. A straight forward modification is an added D-part, the tuning of which exactly parallels that of the PID-regulator. The same parameters $N = 4$ and $T_d = 6\ min$ give almost identical behaviour and robustness, around the crossover frequency, and the tuning of the D-part similarly would benefit from a scheduling with respect to the process time constant T. The main difference as compared to the PID-regulator is that the system is only conditionally stable.

Safety nets have to be included to ensure a minimal gain through the process. The form (6.11)-(6.12) for the regulator is preferred and the phase-lead derivative term should be interpreted as an observer with one more state corresponding to an approximate process time constant T. The safety net, the anti-windup features, and the mode change mechanisms are then more easily designed.

6.5 Discussion

The design of substrate control of yeast fed-batch production has been discussed. The control is based on a broth ethanol signal. The process knowledge described in the previous chapter was used for control design. The effort made on modelling the fast dynamics is not explored fully. Most of the results here are based on the low frequency aspects of the model only, and it is thus approximated with a pure integrator. However, the model of the load disturbance was found important.

The main reason for control is to track the exponential increase in the feed demand during a cultivation. The variation in process dynamics is considered to be of minor importance. Analysis show that introduction of a basic dosage scheme may result in a smaller disturbance and smaller ethanol errors. An observer of the exponential load gives a further improvement of the ethanol control. An error in the initial estimate of the feed demand is eliminated, and influence from variation in the growth rate parameter is reduced. An internal model of the approximate exponential feed demand thus facilitates the low frequency disturbance rejection without altering the properties around the bandwidth.

The sensor response time sets a limit to the strength of the disturbance rejection. A moderate phase advance can be obtained by derivative action. However, tuning of the derivative part of the regulators is found difficult and quite sensitive to the process parameters. From the dynamical studies in chapter five, dead time compensation seems feasible and it would provide more phase advance, but probably require more parameter scheduling. The robustness may thus be sacrificed.

The emphasis of low frequence disturbance rejection and the lack of excitation around the bandwidth suggests that adaptivity should be applied to the internal disturbance model.

7

Control of Continuous Fermentation

There are several aspects of process design to make industrial ethanol production using fermentation a competitive alternative to conventional techniques. There is a strong incentive to develop a continuous process and different reactor configurations have been tested. The control system plays an important role to achieve a high production rate and good utilization of the raw material, despite disturbances in for instance substrate composition and cell condition. The scarcity of sensors is often a limiting factor for control and indirect measurements must be used. However, in this work on-line measurements of substrate and product were available.

This chapter discusses briefly certain trade-offs in process design and what influence these choices may have on control design. The laboratory experimental set-up with a continuous tank and a tube reactor both with immobilized yeast is described and experiences from simple dynamical experiments and control are summarised.

7.1 Continuous fermentation

The major advantage of a continuous process is better utilization of the equipment compared to traditional batch-wise operation. However, there are some technical difficulties to overcome.

A major practical problem encountered in biotechnical processes is to avoid infections. Other microorganisms may grow on the substrate and eventually compete with the original process. The result is a decreased yield and production

rate, and at worst a destroyed product. Continuous operation of a bio-reactor put higher demands on sterility of the equipment than batch-wise operation, where the reactor is cleaned between the runs. A difficulty of more fundamental nature is the question of genetic stability. In batch operation the reactor is restarted with a well defined culture at each run, while in continuous operation the cell culture grow over many generations between restarts. Another area of technical difficulties is separation of the product from the effluent stream. Cells and unconverted substrate should be recycled.

Ethanol fermentation

Ethanol fermentation using yeast is in several respects well suited for continuous operation. There are also work on using a bacteria, *Zyomona mobilis* for ethanol fermentation (Lee and Rogers, 1983). Yeast is a robust microorganism. It can for instance live at lower pH than many others. A pH of about five in the reactor has a good effect on lowering the risk of infections. Further, the cells are comparatively large and there are good possibilities to separate them from the product. There are techniques on industrial scale (Biostil) for continuous separation and recycle of the cells to the reactor. Another approach to keep the cells in the reactor is cell-entrapment. Beads of alginate gel that contains yeast have been extensively studied in the laboratory (Krouwel, 1982; Nunez and Lema, 1987) and also found its way to industrial scale in Japan, the Kyowa Hakko plant (Najashima *et al*, 1984).

Reactor configuration

Several different reactor types have been developed for ethanol production the last decade. However, many design ideas are not realistic for industrial use. It is still interesting to consider the classical reactor types, the tank and the tube reactor.

A continuous stirred tank reactor is a simple construction, but on an industrial scale it may be difficult to obtain homogeneous conditions. The main drawback with the continuous tank reactor is that not all substrate is converted, but follows the product in the outlet stream. In a tubular reactor practically all sugar can be converted, thus a maximal yield of the substrate is feasible. The major difficulty with a tube reactor for ethanol fermentation is the large gas evolution. Carbondioxide is produced in the conversion of sugar to ethanol. It is difficult to make the gas evolve without disturbing the concentration profile in the tube.

It is interesting to try to combine the properties of the tank and the tube reactor (Yamané and Shimizu, 1982; Shama, 1988). This could be done in several ways. In the experimental set-up presented in the next section, a tank and a tube reactor are coupled in series. The yeast is immobilized in alginate beads. The major gas production is in the tank reactor and the residual sugar is converted in the tube. An alternative is the fluidized bed reactor which combines

the properties of a tank and a tube reactor. It has been successfully used on an industrial scale (Najashima *et al*, 1984). In such reactor type the yeast concentration is lower than in a tube reactor and the sugar profile is maintained despite the carbondioxide production.

Downstream processing

It is important to consider the requirements of the subsequent distillation when the control for the fermentation is designed. The cost of distillation increases when the product concentration decreases from the fermentation step. This motivates flow rate through the fermentor as the proper control variable. In this way, the concentration of the feed is kept as high as possible. The alternative, to control the feed concentration by dilution, will in the end give higher distillation costs.

Control objectives

The control objective is to ferment all sugar and maintain a constant ethanol concentration at the outlet. Under these constraints the production rate should be as high as possible. For long time operation it is important to supply minor amount of oxygen for cell regeneration. This is difficult to do without decreasing the anaerobic fermentation processes and thus calls for careful control. However, this question is not further discussed here.

7.2 The laboratory experimental set-up

Continuous ethanol fermentation was studied at a laboratory scale. A continuous stirred tank reactor was used in combination with a tube reactor. Different configurations were tested. Throughout the yeast was immobilized in beads of alginate gel. The idea was to study in what way on-line measurement of substrate and product concentrations could be used for control of the process.

The experimental set-up is shown in Figure 7.1. The tank reactor had a volume of about 5 litres and was filled with yeast alginate beads which gave an effective liquid volume of 3.5 L. The tube reactor was packed with beads and had an effective liquid volume of about 1.5 L. The ratio between the length and the diameter of the tube was roughly fifteen to one. Further details are given in (Mandenius *et al*, 1987). Experiments were done with each reactor separately and also in combination. The sizes of the tank and the tube were chosen so that the tank reactor left no more sugar to the tube reactor to convert than the gas evolution in the tube permitted and that all residual sugar was converted in the tube. The flow through the tank-tube serie was the main control variable, and deviation from the nominal flow rate changes the balance of activity between the two reactors. If the flow rate is increased, the concentration of the residual sugar from the tank increases and the activity is thus moved towards the tube reactor.

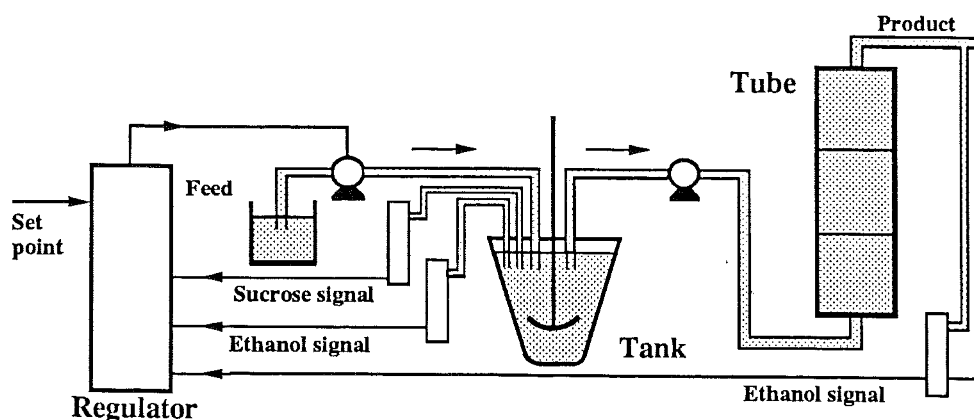


Figure 7.1 Experimental set-up for continuous ethanol fermentation.

On-line measurements of substrate and product were done using two different techniques. The substrate concentration, sucrose, was measured using an enzyme thermistor (Danielsson *et al*, 1981). The ethanol concentration was measured using a gas permeable membrane in combination with a semiconducter based sensor (Mandenius *et al*, 1987). Both measurements had a time delay of a few minutes due to transport.

The flow rate through the process was the main control variable. Control by changing the substrate concentration to the tube reactor was also tried as a complement.

7.3 Experience from the laboratory

Several different combinations of reactors and controllers were tested. Most work was done on the tank reactor, but experience with the tube- and the tank-tube reactor combination are also shown. The emphasis in this section are on recorded transients in substrate and product concentrations that occurred under feedback, and also results from simple open-loop experiments. The presented data gives an idea of what part of the process dynamics that can be considered well-known and what part that is more difficult to understand and perhaps calls for further research if certain control objectives should be met.

Tank reactor

It is straight forward to formulate a dynamic model of the tank reactor based on mass balance equations. The transport of substrate and product in the alginate bead was considered fast and such dynamics could be neglected. Further, the cell growth is hampered when the cells are immobilized. Therefore the catalytic activity of the reactor could be regarded as constant. Variations are in the time scale of ten hours and are treated as slow parameter variations.

Mass balance for the reactor with the sugar and ethanol concentrations S and E respectively, and the flow rate F gives

$$
\begin{aligned}
\frac{dS}{dt} &= -kS + \frac{F}{V}(S_{in} - S) \\
\frac{dE}{dt} &= pS - \frac{F}{V}E
\end{aligned}
\tag{7.1}
$$

where the incoming feed has the sugar concentration S_{in} and the reaction kinetics are described by k and p. The volume V refers to the liquid volume of the reactor. Since the alginate beads take up some space the total volume is considerable larger.

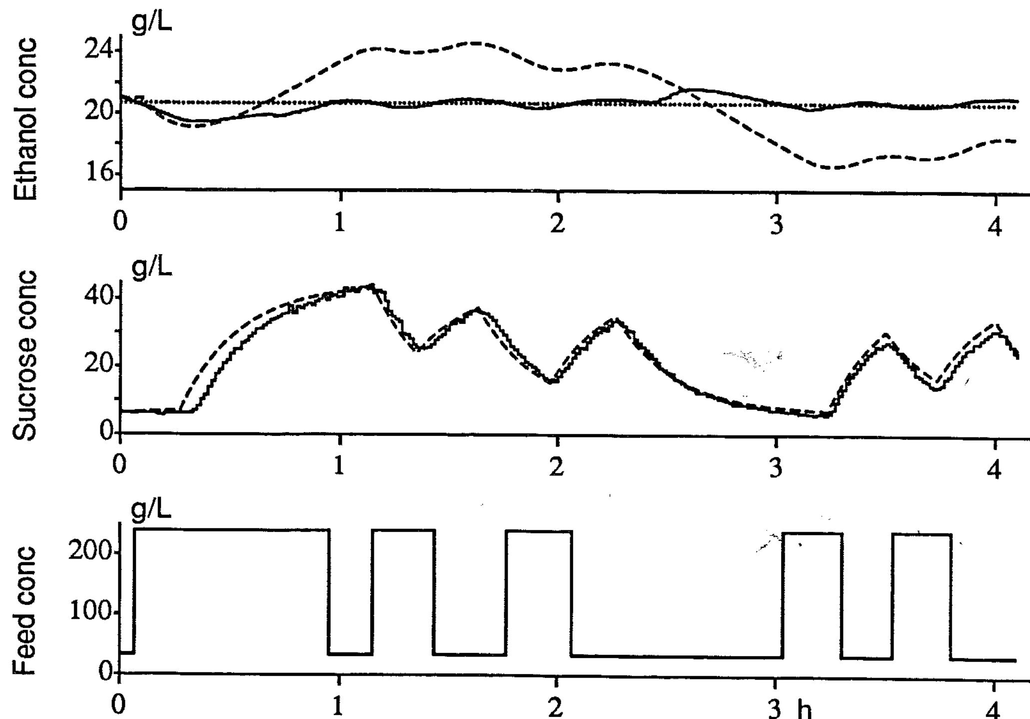


Figure 7.2 Dynamic experiment in closed loop with the tank reactor. The solid lines are measured data and the dashed lines are results from simulation of the model (7.1).

The model (7.1) was found adequate for control design. Simple experiments were done to determine the parameters. In Figure 7.2 is shown a dynamic experiment in closed loop. The flow rate was kept constant but the incoming substrate concentration was changed between two levels using relay feed-back from the ethanol signal. The measured ethanol and sucrose signals are compared with the model using simulation. The model describes the sucrose level well. The simulated ethanol signal seems to approximately follow the rapid changes in the control variable, but there is also a slow mode that is not captured. This is natural since the ethanol dynamics contains a slow mode related to the space time V/F of the reactor.

The main drawback with the model (7.1) is that under certain conditions intermediate sugars, glucose and fructose, are accumulated in the reactor. The disaccharide sucrose is cleaved extracellularly to glucose and fructose. Normally, the glucose and the fructose molecules are taken up immediately by the cells, but under certain conditions this uptake does not meet the rate of sucrose cleavage.

The model (7.1) was successfully used for different control designs. The measurement signals contained time delays of about 5-10 min and the process model was used to obtain regulators with time delay compensation. In Figure 7.3 and Figure 7.4 is shown flow rate control of the sucrose concentration using observer based time delay compensation (Axelsson *et al*, 1982). In Figure 7.3 is shown the response to a set-point change. Note that most of the control action comes before changes in the measured signal. The robustness of the control system to an impulse disturbance was tested in Figure 7.4. Water was poured into the reactor and the regulator was found to quickly compensate for this disturbance and there were no signs of unstable control actions.

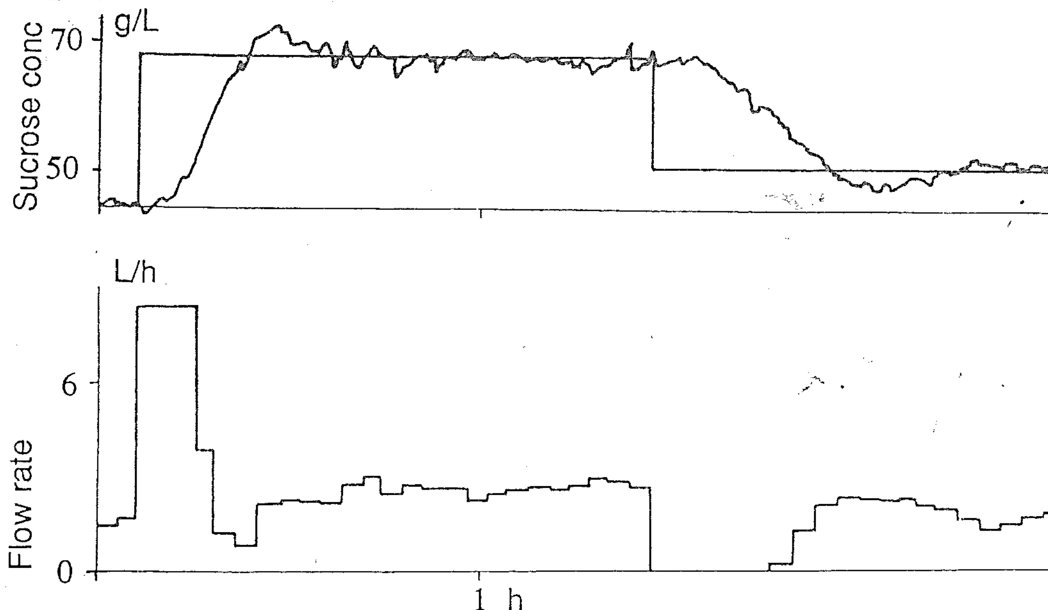


Figure 7.3 Control of the sucrose concentration. Change of set-point.

In another series of experiments the product concentration was controlled instead (Mattiasson *et al*, 1982). Ethanol was measured on-line with a similar time delay as for the sucrose sensor. In Figure 7.5 compensation of an impulse disturbance is shown, similar to the disturbance in Figure 7.4. Water was poured into the reactor and the regulator brought the ethanol concentration back to the set-point within half an hour. The reactor was still in a transient. This is seen from the slow recover of the sucrose concentration. In Figure 7.6 is shown the response to change of set-point. The lower diagram shows how the state moves in the state space. It follows almost a line.

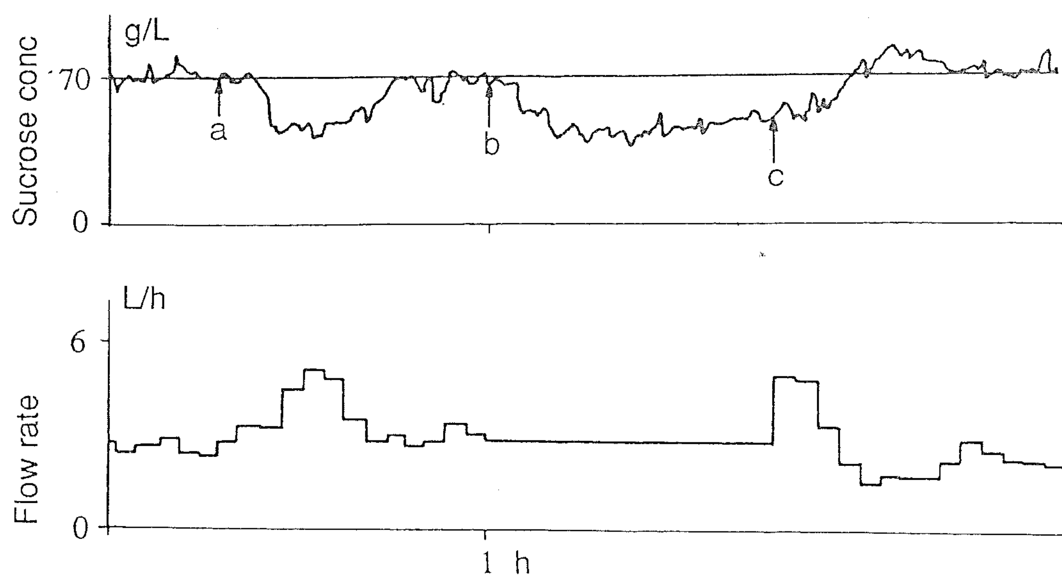


Figure 7.4 Control of the sucrose concentration. Elimination of a disturbance. Water was poured into the reactor at time a. At b the regulator was turned off and the disturbance was repeated. At c the regulator was turned on again.

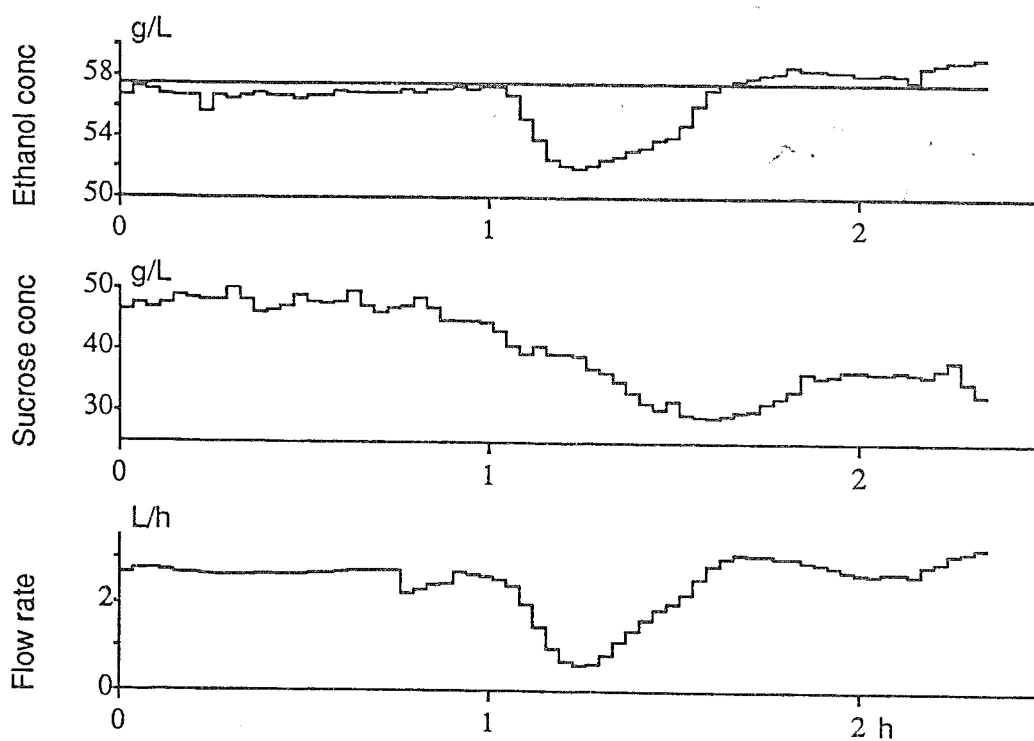


Figure 7.5 Control of the ethanol concentration. Elimination of a disturbance.

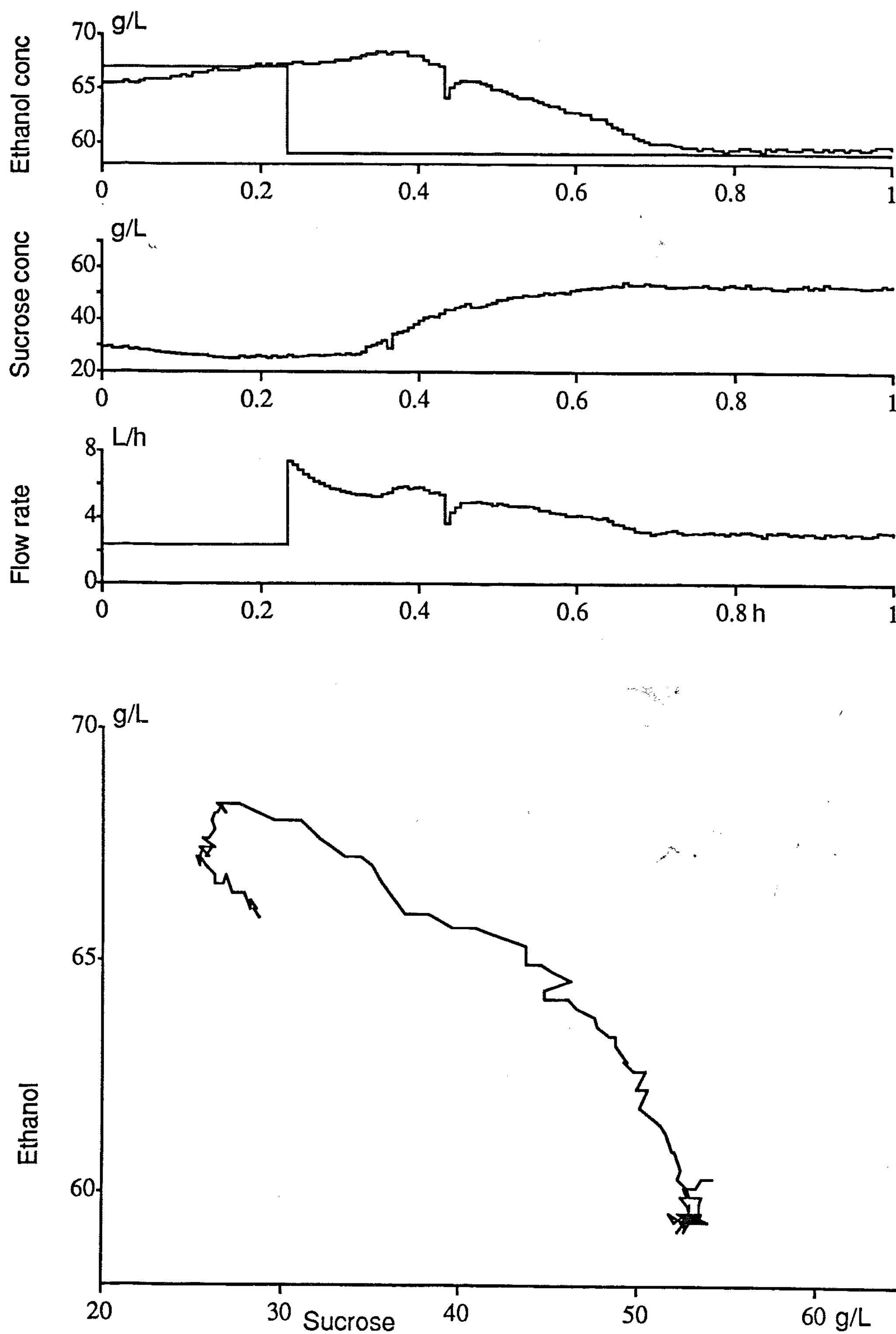


Figure 7.6 Control of the ethanol concentration. Change of set-point. The lower diagram shows how the state moves in the state space. The sucrose and ethanol signals are here slided to compensate for the difference in time delay. The outlier after 0.45 h is also removed.

Tank and tube reactor in combination

The main reason to introduce the tube reactor was that the residual sugars should be converted to ethanol and no substrate be wasted. Further, the production rate of ethanol increases with the flow rate and therefore it should be kept as high as possible. The limiting factor is the rate of carbondioxide production in the tube. In Table 7.1 are shown stationary values of sugar and ethanol concentrations at different flow rates.

Table 7.1 Residual sugar concentration at three different flow rates.

Flow rate [*L/h*]	*Sugar*	*Tank* [%]	*Tube* [%]
0.9	*Sucrose*	7	0
	Glucose	12	4
	Fructose	17	0
	In total	36	4
1.8	*Sucrose*	21	0
	Glucose	23	25
	Fructose	29	39
	In total	73	64
2.4	*Sucrose*	25	9
	Glucose	27	25
	Fructose	27	34
	In total	79	68

The experience from these stationary evaluations was that the intermediate metabolites glucose and fructose rose to high concentrations in both the tank and the tube reactor. However, it was difficult to measure these sugars on-line. Therefore the control objective to convert all sugars was formulated in terms of a maximal, or high, ethanol concentration at the outlet of the tube reactor.

In order to improve the control of the outlet ethanol concentration, the ethanol signal from the tank reactor was also used. Cascaded PID control was chosen because it was believed to be simple to tune at the process. Proportional control was used for the inner loop and the outer loop had both integral and derivative parts as well. The structure of the control system is shown in Figure 7.7. The regulator were tuned at the process. The regulator gain for the inner loop was five times higher than for the outer loop. The integral time was chosen around 0.3 h without stability difficulties. The derivative part was difficult to tune and was eventually turned off.

The process was run for a couple of weeks and different regulator parameters were tested and simple dynamical experiments were done. In Figure 7.8 control of the outlet ethanol concentration is shown. The process was run over almost two days. The activity of the cells changed in the time scale of ten hours and the regulator adjusted the flow rate so that the outlet ethanol concentration

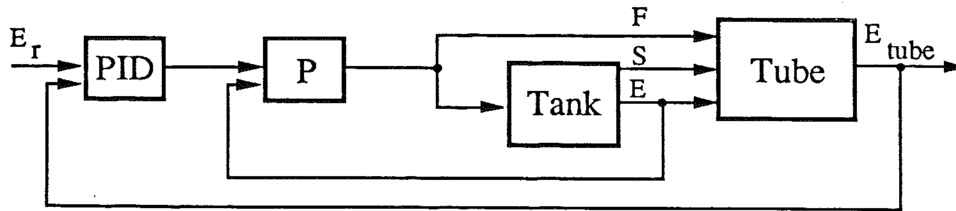


Figure 7.7 Block diagram of the cascaded PID regulator structure for the tank- and tube reactor combination.

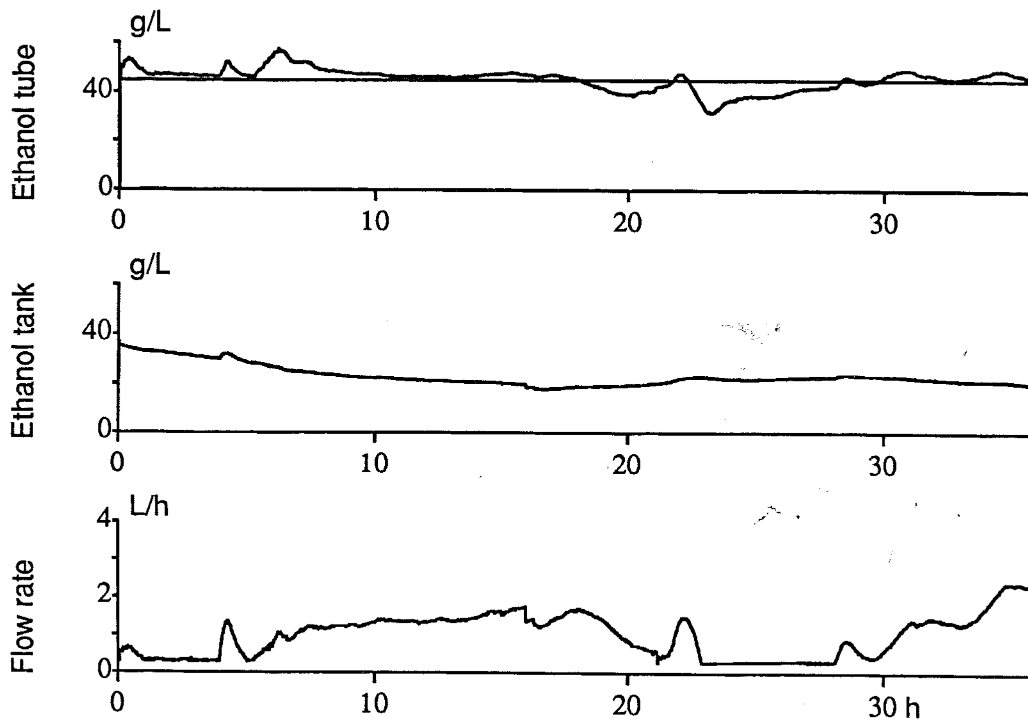


Figure 7.8 An example of control of the outlet ethanol concentration.

remained close to the set-point. The variations of cell activity was likely due to temperature variations in the reactors. The process variables were close to limitations in this experiment. The control variable saturated during the first few hours and also a few hours between twenty and thirty hours from start. The regulator behaved well. The minimal flow rate was chosen non-zero for reasons of process design. The outlet ethanol concentration was close to the stationary maximal concentration, An estimate of the maximal concentration that could be obtained is 65 g/L (1.4 mol/L), since the feed sucrose concentration was 120 g/L (0.35 mol/L) and maximal conversion of sucrose gives four molecules of ethanol.

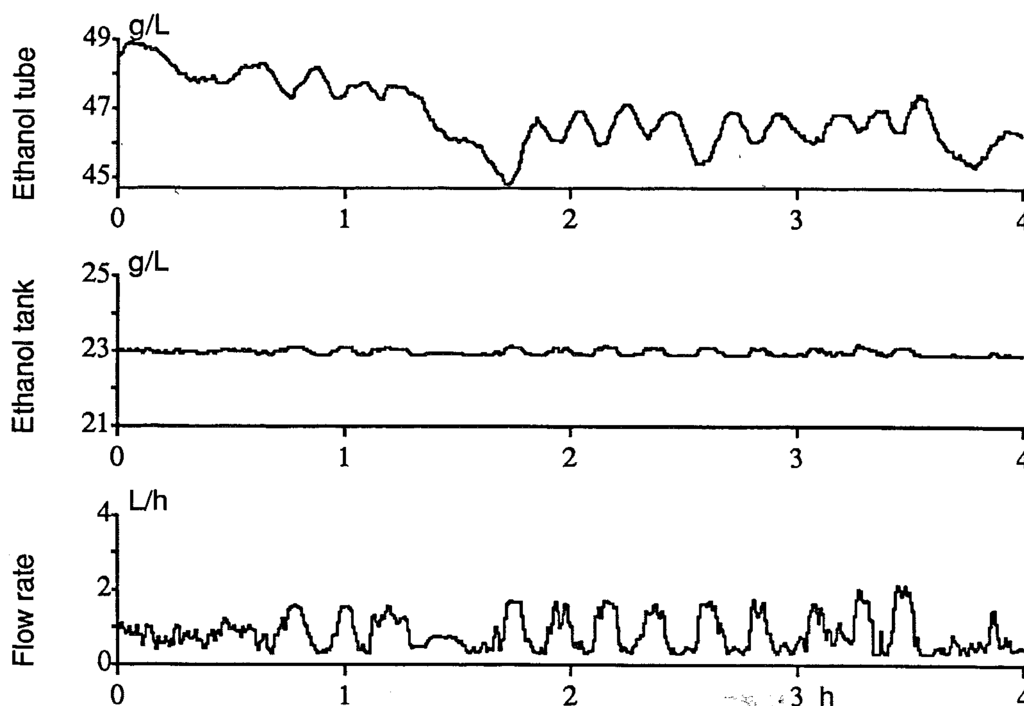


Figure 7.9 Fast dynamics of flow rate controlled tube reactor.

The tube reactor has a potential of a complex dynamical behaviour. It is evident that a tube reactor has slow dynamics due to transport through the tube. However, in Figure 7.8 fast variations were recorded at the outlet ethanol concentration and in Figure 7.9 rapid dynamics between changes in the flow rate and the outlet concentrations were found. Bad tuning of the regulator parameters had given an unstable control system. In order to better understand the dynamics of the tube a simple experiment was done with the tube reactor separately. The flow rate was kept constant and step changes were made in the inlet sucrose concentration. The result is shown in Figure 7.10. The flow rate was 1.1 L/h which gives a space time of about 45 min.

Discussion

Experience has been presented from various experiments with feedback control of laboratory scale tank- and tube reactors, based on on-line measurement of substrate and product concentrations. The sucrose and ethanol sensors were found useful for process control. Process dynamics showed to a large extent a deterministic behaviour. Under certain conditions, however, considerable amounts of intermediate compounds in the fermentation accumulated. These compounds were glucose and fructose, and they were difficult to measure on-line.

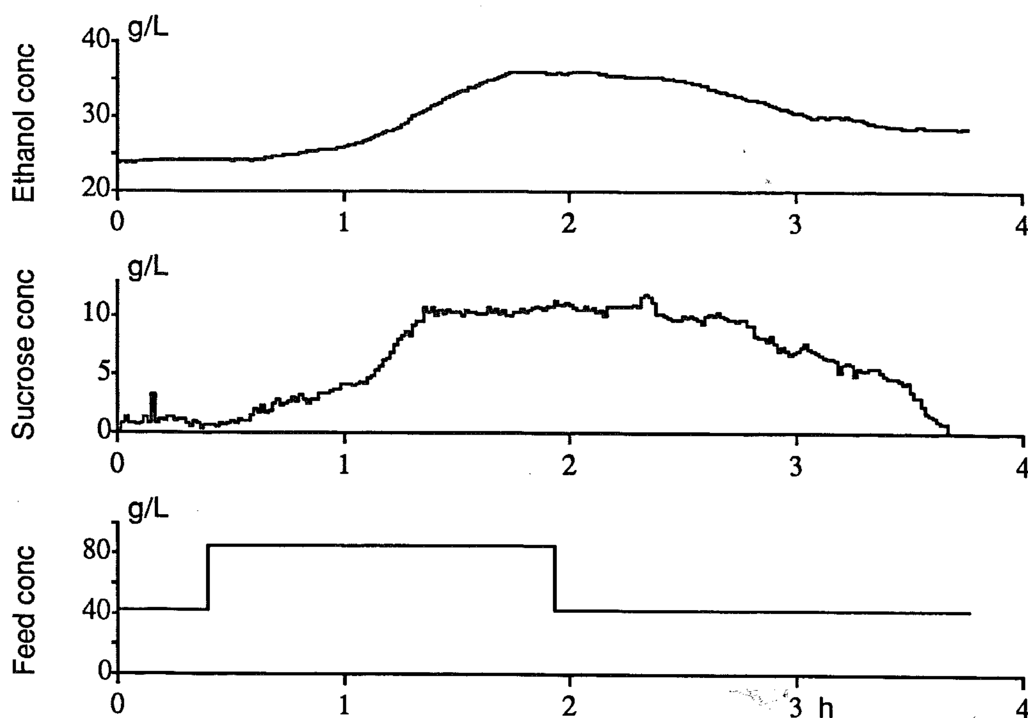


Figure 7.10 Step change in the inlet sucrose concentration to the tube reactor. The flow rate F was kept constant at 1.2 L/h.

The dynamics of the tank reactor was well described by a simple model and control design based on this model was used for time delay compensation. The flow rate was chosen as control variable. A comparatively high bandwidth could be obtained without stability problems, both for substrate control and for product control. In Figure 7.4 the disturbance was eliminated in 20 min, while without control it would take about 2 hours.

The combination of the tank and the tube reactors was more difficult to control. The flow rate through the reactor was chosen as control variable. The outlet ethanol concentration from the tube was controlled using a cascaded PID regulator based on ethanol measurement in the tank and at the tube outlet. The regulator was tuned at the process, not without difficulties. The derivative part of the outer loop was difficult to use because the process dynamics contained also a rapid mode.

The control objective of the tank- and tube process was to ferment all sugar and maintain a high productivity. Ideally, the outlet sugar concentrations should be measured and the flow rate adjusted to give zero residual sugar concentration. Here, the outlet ethanol concentration was measured instead and the control objective was reformulated to keep maximal ethanol concentration, or at least a high concentration. Both control objectives implies an important non-linearity.

The sensor signal can only give information about incomplete conversion, not that the conversion rate is too low. However, this asymmetry was not evident in the experiments, since the ethanol set-point was chosen somewhat lower than the maximal value. By placing the sensor inside the tube at a short distance from the outlet this problem is avoided.

The tube reactor has a potential for a complex dynamical behaviour. The tube has a slow dynamical response due to transport through the column. It was also found, however, that the outlet ethanol concentration changed almost immediately for large changes in the flow rate. The physical background to this phenomena is not clear. It is likely due to a displacement of the the sugar and ethanol profile relative to the immobilized cells. If this profile change produces a change of substrate at the end of the tube this would also give a fast change in the ethanol concentration at the outlet.

Better control may be achieved by better understanding of the process dynamics. The accumulation of intermediates glucose and fructose, and also the rapid dynamics of the tube, are important to understand for better process control. On the other hand, the control authority is limited and ways to modify the process configuration may be equally important for good process control. One example is that the inlet flow to the tube could be mixed with a stream directly from the feed vessel. In this way a higher substrate concentration could quickly be obtained at the inlet and the flow rate through reactors could slowly be adjusted in order not to disturb the concentration profiles in the tube. However, in this configuration it is difficult to avoid a buffer tank between the tank and the tube reactor.

There are several ways to continue this work, and maybe also change the reactor configuration. Here, the choice was made to take a closer look at the non-linear properties of the tank reactor. That work is presented in the next chapter.

8

Non-linear Control Design

In this chapter is discussed one aspect of control of a continuous tank reactor that gives a simple example of the difficulties with control of non-linear process dynamics. The process considered is the ethanol fermentation from sugar using immobilized yeast, which was discussed in the previous chapter. The process is controlled using variations in the flow rate. On-line measurement of the product as well as substrate concentration are assumed available. The discussion is focused around control of the product concentration. PID-control based on ethanol measurement is investigated for set-point control as well as for disturbance rejection. Mathematical analysis shows that under certain transient conditions in the reactor, information about the substrate concentration is important for good control. In these situations the non-linear character of the process is important to consider. A time optimal control problem is formulated and solved using dynamic programming. The resulting control strategy is simple and easy to understand. For large disturbances it gives a control strategy with change of flow rate in the reversed direction from a conventional PID-regulator. Attempts were also made to apply techniques based on differential geometry concepts for analysis and control design.

8.1 Analysis

The process model for the continuous tank reactor, presented in the previous chapter, will be analysed in this section with respect to stationary points and general dynamic behaviour.

Process model

Recall the equations for the sugar and the ethanol concentrations in the tank reactor, S and E respectively,

$$\begin{cases} \dfrac{dS}{dt} = -kS - \dfrac{1}{V}FS + \dfrac{1}{V}FS_{in} \\ \dfrac{dE}{dt} = pS - \dfrac{1}{V}FE \end{cases} \tag{8.1}$$

where S_{in} is the feed sugar concentration, V is the broth volume and k and p are kinetic constants.

Normalization of the process model

To simplify further analysis and control design the process model is normalized. The following transformation

$$\begin{cases} S' = S/S_{in} \\ E' = kE/(pS_{in}) \\ F' = F/(kV) \\ t' = t/k \end{cases} \tag{8.2}$$

gives the state equation

$$\begin{cases} \dfrac{dS'}{dt'} = -S' + (1 - S')F' \\ \dfrac{dE'}{dt'} = S' - E'F' \end{cases} \tag{8.3}$$

Subsequently these normalized process variables will be used and the prime index will be left out.

Stationary points

A constant flow rate F^o results eventually in steady state concentrations S^o, E^o of substrate and product in the reactor. The time it takes is related to F^o/V; the space time in chemical engineering terms. The steady state values for $F^o > 0$ are

$$\begin{cases} S^o = \dfrac{F^o}{1 + F^o} \\ E^o = \dfrac{1}{1 + F^o} \end{cases} \tag{8.4}$$

The response of sugar and ethanol concentrations to different flow rates is shown in the Figure 8.1. The initial concentrations are zero. Note that $dS^o/dF^o > 0$ while $dE^o/dF^o < 0$. Note also that stationary state is reached faster at higher

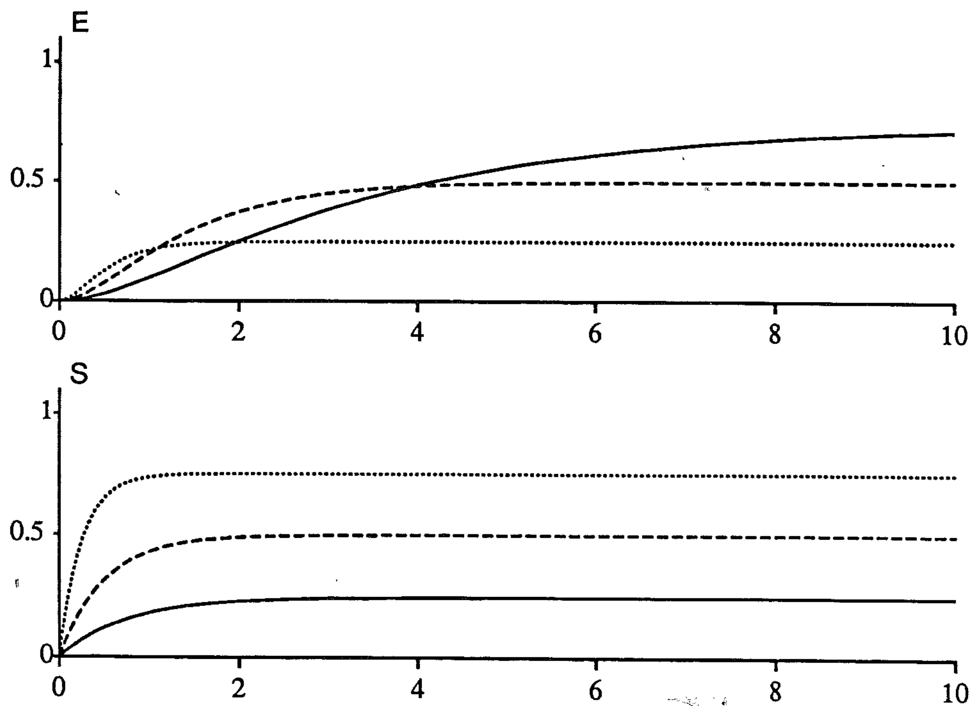


Figure 8.1 Step response for three different flow rates.

feed rates. The stationary points can be plotted in the phase plane. Changing the stationary flow rate generates a line of stationary points.

$$E^o + S^o = \frac{1}{1+F^o} + \frac{F^o}{1+F^o} = 1 \tag{8.5}$$

This line is here referred to as the equilibrium line. It corresponds to the asymptotic reaction invariant of the process (Fjeld *et al*, 1974; Waller and Mäkilä, 1981). The start-up trajectories in the phase plane for different constant flow rates are shown in Figure 8.2.

Convergence to the stationary points

The stationary points form an equilibrium line in the state space. Consider the projection P of the trajectories to a line orthogonal to the equilibrium line

$$P = 1 - S - E \tag{8.6}$$

The differential equation for P becomes

$$\frac{dP}{dt} = S - (1-S)F - S + EF = -PF \tag{8.7}$$

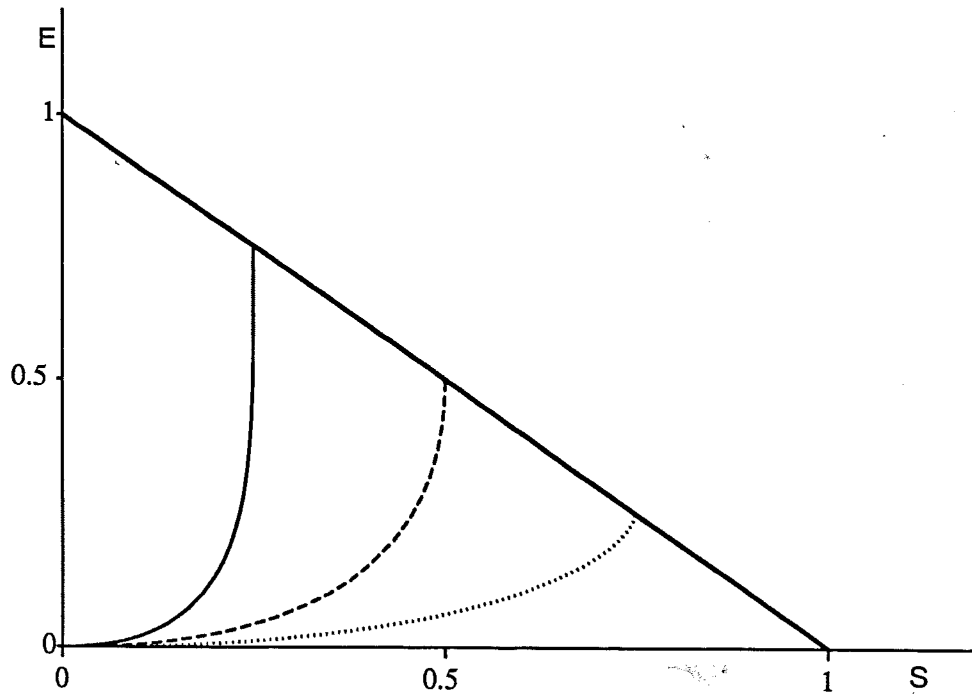


Figure 8.2 The state reaches the equilibrium line for different constant flow rates.

From the last equation (8.7) it is clear that the state converges to the equilibrium line and that there is no way to reverse this process, because the flow rate is a non-negative quantity. However, variations in the flow rate change the rate of convergence.

Choice of coordinates and integration of the equations

The projection (8.6) reveals a simplicity of the process dynamics. The subsequent analysis will certainly be clearer in the coordinates

$$\begin{cases} x_1 = S \\ x_2 = P = 1 - S - E \end{cases} \tag{8.8}$$

which gives

$$\begin{cases} \dfrac{dx_1}{dt} = -x_1 + (1 - x_1)F \\ \dfrac{dx_2}{dt} = -x_2 F \end{cases} \tag{8.9}$$

Integration of the state equation is also simplified in these coordinates. The

technique with integrating factor gives

$$\begin{cases} x_1(t) = e^{-(t-t_0)}\phi(t,t_0)x_1(t_0) + \int\limits_{t_0}^{t} u(\tau)e^{-(t-\tau)}\phi(t,\tau)d\tau \\ x_2(t) = \phi(t,t_0)x_2(t_0) \end{cases} \tag{8.10}$$

where $\phi(t,t_0) = \exp\{-\int\limits_{t_0}^{t} u(\sigma)d\sigma\}$. A constant control variable $u(t) = u^\circ$ gives

$$\begin{cases} x_1^\circ(t) = x_1(t_0)e^{-(1+u^\circ)(t-t_0)} + \frac{u^\circ}{1+u^\circ}\left(1 - e^{-(1+u^\circ)(t-t_0)}\right) \\ x_2^\circ(t) = x_2(t_0)e^{-u^\circ(t-t_0)} \end{cases} \tag{8.11}$$

A simple back transformation will give the solution in the original physical coordinates $S(t)$ and $E(t)$.

Remarks on parameter variations

Consider the equation for the equilibrium line (8.5) and translate it to the original physical coordinates

$$pS + kE = pS_{in} \tag{8.12}$$

The kinetic parameters k and p may vary slowly but this will only alter the slope of the equilibrium line. Changes in the feed substrate concentration S_{in} moves the line in a parallel direction. Such a change may come abruptly due to changes of batch. After such a disturbance the state, originally in stationarity, is far from the equilibrium line and the mode (8.7) is excited.

8.2 Control objectives

The control objective of the fermentation process is here chosen as to control the product concentration in the reactor. The control variable is the flow rate and it may vary over a wide range. Under these conditions non-linear dynamics of the tank reactor are important to consider.

Several different control objectives are possible for control of a continuous fermentation process. Here, an objective is identified that will focus control design on non-linear process dynamics. Control of the product concentration should be important for downstream processing. The residual substrate may be recirculated, and at least in such a case transients of high substrate concentration to keep the product concentration within prescribed range, might not be bad economy at all.

8.3 Limitations of linear control design

In the previous chapter, section 7.3, an account was given of the experience from control of the tank reactor. Both set-point control and elimination of small disturbances were tested with good results. In principle, a linear regulator was used. However, the sensor had a time delay, and the linear control design was actually based on the predicted measurement signal from the process model (7.1). The limitations of linear control design will here be discussed, disregarding the sensor time delay.

Set-point control

The experience from the laboratory was that set-point control of the product concentration was similar to substrate control. Both substrate and product concentrations seemed to have the same dynamics. This observation is easily understood from the analysis in Section 8.1. Recall that after a start-up transient (8.7) the substrate and product concentrations are proportionally related

$$E = 1 - S$$

irrespective of subsequent variations in the feed rate. Thus, the dynamics between variations in the flow rate and the product concentration is of first order, but note that it is still non-linear. However, the dynamics can be viewed as a linear system with a variable gain. The gain variations can easily be accounted for by gain-scheduling of the regulator.

In Figure 8.3 is shown a simulation of PI-control of the product concentration. The response to a change of set-point is illustrated. The trajectory in the state space is also shown.

Disturbance rejection

Response of the control system to a disturbance was tested in the laboratory and the system behaved satisfactorily. However, a more careful investigation shows that a larger disturbance is eliminated very slowly by a PI-regulator. In Figure 8.4 is shown a simulation of PI-control, where compensation of a small and a large disturbance are compared. The trajectories in the state space are also shown.

It is easy to understand why it takes such a long time to eliminate the large disturbance in Figure 8.4. The regulator acts in the same way as for the small disturbance. The only difference is that the control action is correspondingly larger. This results in a very low flow rate and from (8.9) it is clear that the rate of convergence to the stationary point is proportional to the flow rate. Note, that if the disturbance is large enough, the PI-regulator will give zero flow rate. If the substrate concentration in the tank is not high enough, then a P-regulator would shut off the flow rate permanently.

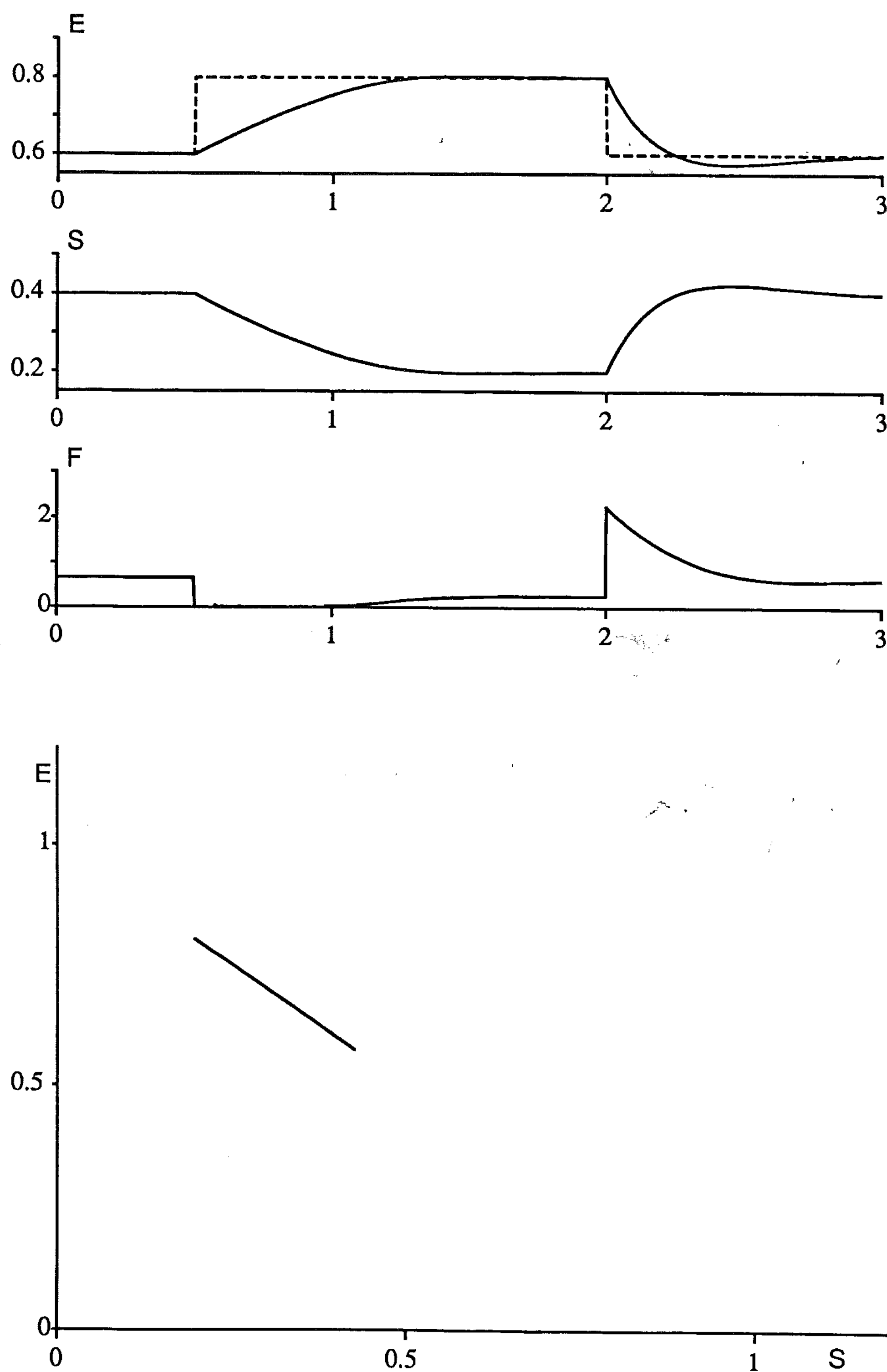


Figure 8.3 Simulation of PI-control of the product concentration. The lower diagram shows the trajectory in the state space.

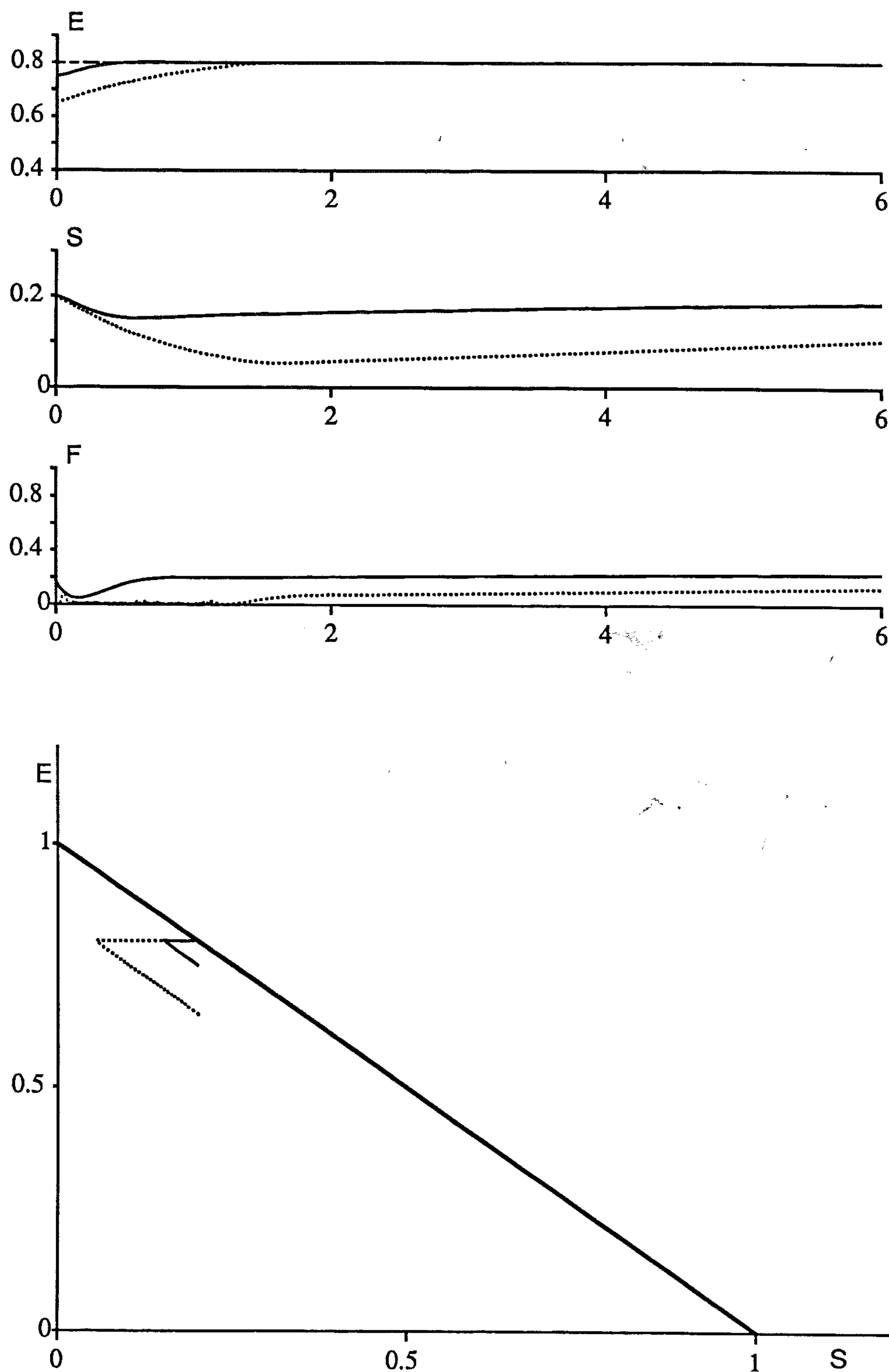


Figure 8.4 Simulation of PI-control of the product concentration. The responses are shown for a small and a large disturbance, respectively.

Linear state feedback

The previous discussion was limited to PID-control of the product concentration, but the limitations are also valid for linear state feedback. For set-point control there is no reason to include measurement of the substrate concentration, since it is proportional to the product concentration. The disturbance rejection could be improved by adding information about the substrate concentration. The idea would be to let the flow rate decrease with decreasing product concentrations but increase with the distance to the equilibrium line. In this way it can be ensured that the flow rate is kept non-zero when both product and substrate concentrations are low. Thus, the control system will bring the process back to the working point. Simulations show, however, that the transient behaviour close to the working point is then not satisfactorily. Small disturbances takes much too long time to eliminate in this way.

The state feedback control law can be visualized as a surface in the space of the two states and control signal. A linear control law implies that the surface is a plane. The idea to account for the distance of the state to the equilibrium line means that the plane is tilted around the equilibrium line. The limitations of linear state feedback compared to general state feedback discussed in the subsequent sections are easily understood in this way.

8.4 Time optimal control design

It was found that PID control of the ethanol concentration could handle set-point changes and compensate for minor disturbances. For larger disturbances that move the state out from the equilibrium line, PID-control gives a very slow recover to the set-point, and for the start-up procedure the PID control could not be used at all. Around a stationary working point the process gain is negative, i. e. to increase the ethanol concentration the flow rate should be decreased. However, during the start-up procedure from zero concentrations in the reactor, the flow rate must be non-zero. In order to quickly build up the concentrations in the reactor the flow rate could be set to a high level and then decreased to the operating flow rate. It is reasonable to believe that such a flow rate strategy is sound not only for zero reactor concentrations but also for certain combinations of low substrate and product concentrations. This means that for small and large disturbances from the equilibrium line reversed control strategies may be needed.

This idea of reversed control strategies will be further investigated below. First a time-optimal problem is formulated and solved and a bang-bang control law is obtained.

Problem formulation

Given the initial state (S_0, E_0), calculate the minimum time strategy to get to the line $E = E_r$ using a bounded insignal $0 \leq u(t) \leq u_m$ where $E_r > 1/(1 + u_m)$.

The resulting control law

The regulator that gives time optimal control makes use of both the ethanol and the sugar measurement signal. The controller has a simple structure. It is of bang-bang type. There are three regions in the state space separated by switching lines where the flow rate is changed between extreme values. In Figure 8.5 is shown the start-up of the process using the time optimal strategy. The ethanol concentration quickly reach the set-point. However, the process is still in a transient. The state moves slowly to the stationary point on the line $E = E_r$. At the switch line $E = E_r$ the bang-bang regulator comes into a chattering mode. In the simulations this is avoided by changing the control law to $u(t) = S(t)/E_r$ when the line $E = E_r$ is reached.

It is interesting to see how the time optimal regulator eliminates small and large disturbances, cf. Figure 8.6. The small disturbance is quickly compensated by setting the flow rate to zero. For the large disturbance, however, the time optimal strategy is to first use maximal flow rate and then zero flow rate. Thus, a reversed strategy is called for. For comparison the behaviour of the P-controller is shown in Figure 8.7 for the same disturbances. Note the difference for the large disturbance. It takes considerable time for the ethanol concentration to come back to the set-point using proportional control.

The result that the bang-bang regulator is time optimal is formulated as a theorem.

THEOREM 8.1

The minimum time to reach the line $E = E_r$ from any point in the first quadrant in the state space for the system (8.3), is obtained by using the bang-bang control law

$$u = \begin{cases} u_m, & \text{if } E_r < E,\ 0 \le S \\ 0, & \text{if } E_r - E_r S < E < E_r,\ 0 < S \\ u_m, & \text{if } 0 \le E < E_r - E_r S,\ 0 \le S < (E_r - E)/E_r \end{cases} \tag{8.13}$$

Proof: The system of differential equations (8.3) has a unique solution for a piece-wise continuous control signal $u(t)$, and the loss function time, is additive. Therefore the method of dynamic programming can be applied. It is natural to consider level curves in the state space of the loss function for constant input signal $u(t) = u$ and from these develop the solution. The level curves, called isochrones, combines points of equal distance in time to the end-points. These isochrones are simple to calculate in the case of constant input. The general solution of (8.3) for $u(t) = u$ will be used several times subsequently. It is

$$\begin{cases} S(t) = S_0 e^{-(1+u)t} + \dfrac{u}{1+u}(1 - e^{-(1+u)t}) \\ E(t) = 1 - S(t) - (1 - S_0 - E_0)e^{-ut} \end{cases} \tag{8.14}$$

Further, the stationary solution (8.4) will be used in different ways to estimate

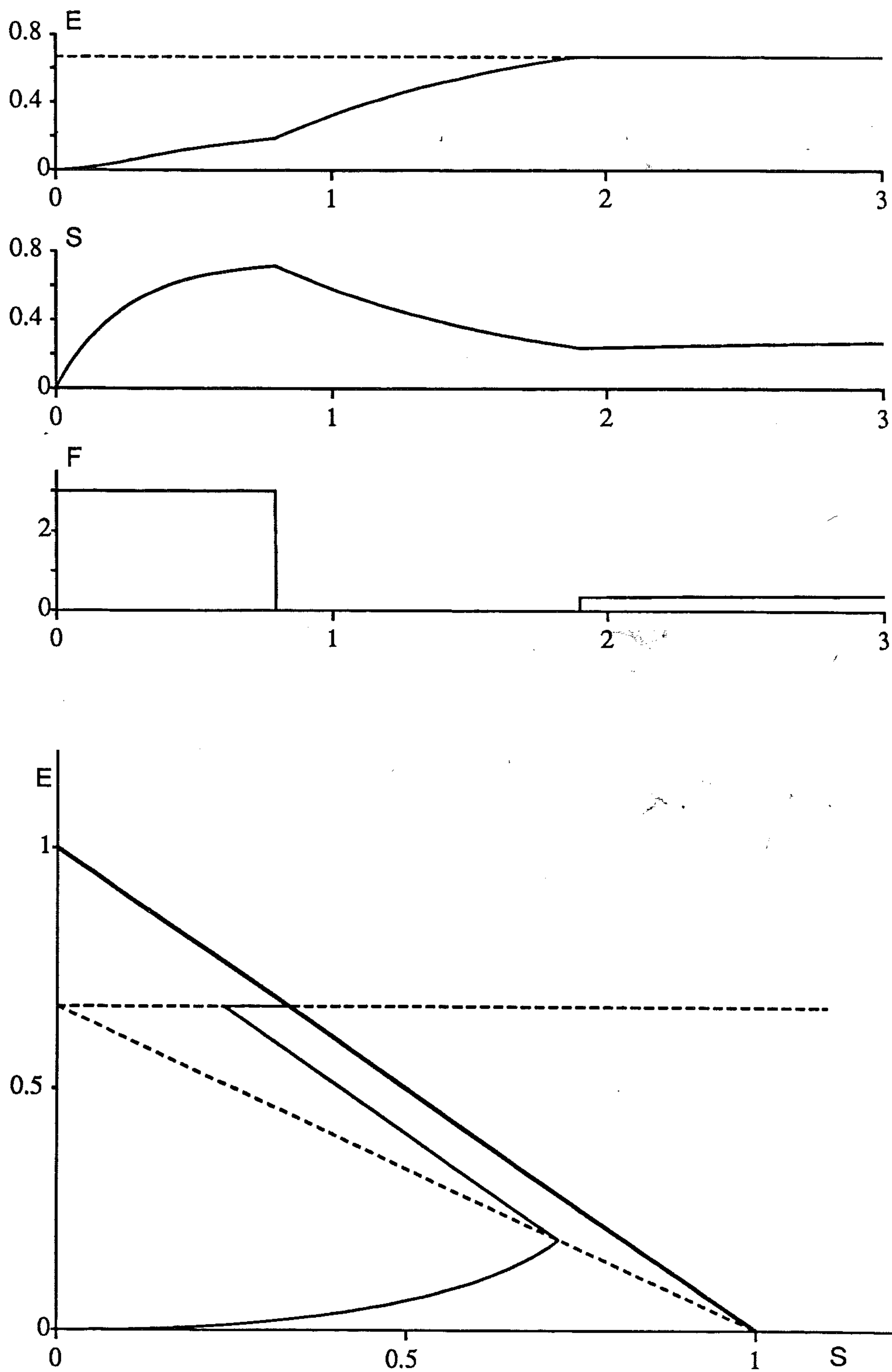


Figure 8.5 Time optimal control applied to the start-up problem. The lower diagram shows the trajectory in the state space.

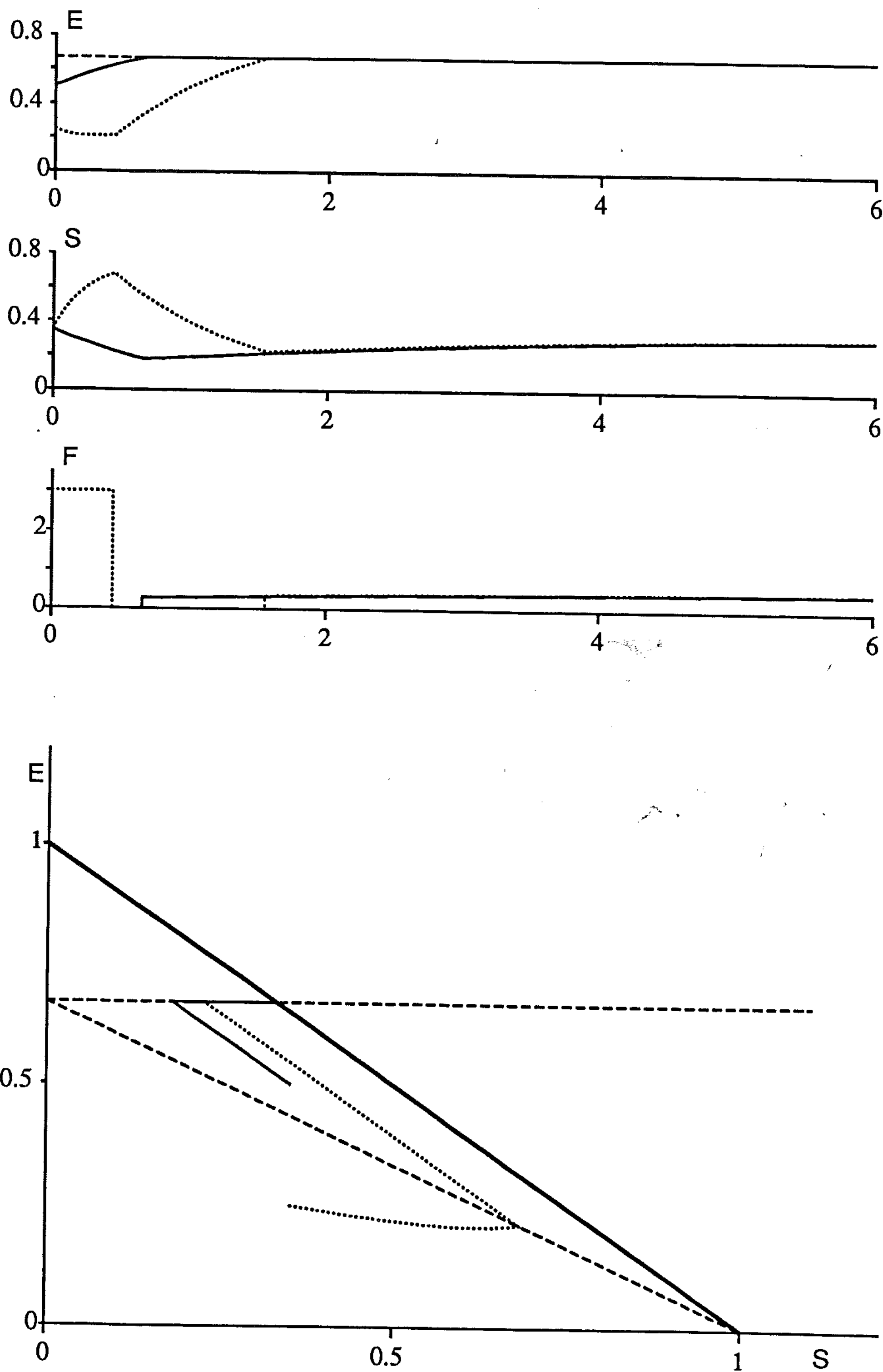


Figure 8.6 Simulation of the time optimal strategy to compensate one small and one large disturbance to the reactor. Below, the trajectory in the state space is shown.

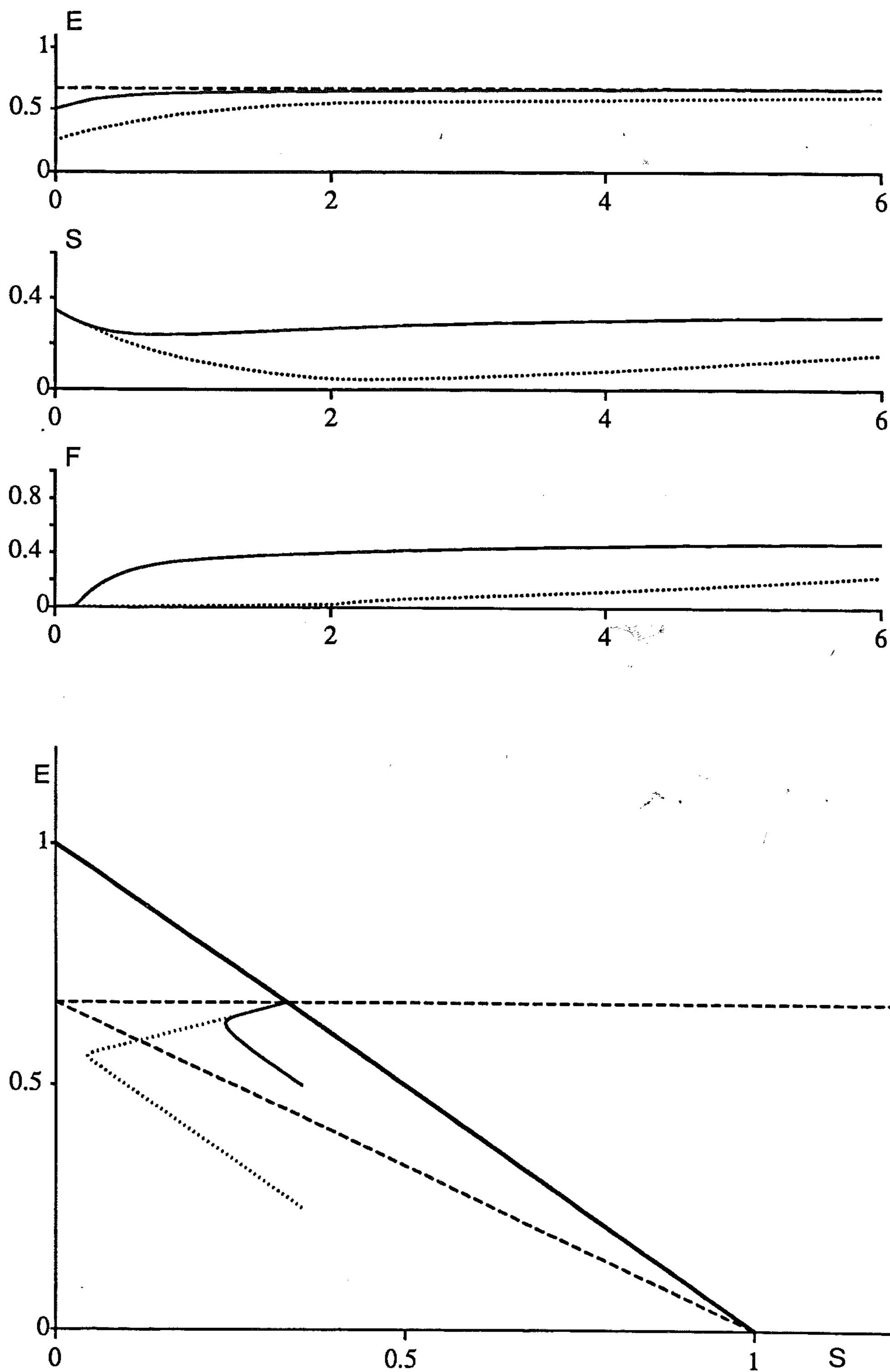


Figure 8.7 Simulation of the process with a proportional regulator. Compensation of one small and one large disturbance is shown. Below, the trajectory in the state space.

certain bounds. The flow rate that gives the desired ethanol concentration E_r and the corresponding sugar concentration S_r in stationarity is denoted u_r.

Consider the area $E_r < E$ in the state space. For states infinitesimally close to $E = E_r$

$$\frac{dE}{dt} = S - Eu < S_r - E_r u = \frac{u_r - u}{1 + u_r} < 0, \quad u > u_r$$

and together with $\partial^2 E/\partial u \partial t < 0$ implies that u should be chosen as large as possible when the state is close to the border line $E = E_r$. Therefore, consider the trajectories in the area for the constant control signal $u(t) = u_m$. Combine points in the state space that are on the distance τ in time from the line $E = E_r$. Simple calculations using (8.14) show that the isochrones form straight lines

$$\begin{aligned} E &= -k(\tau)S + l(\tau) \\ k(\tau) &= 1 - e^{-\tau} \\ l(\tau, u_m) &= 1 - (1 - E_r)e^{\tau u_m} + \frac{u_m}{1 + u_m}(e^{\tau u_m} - e^{-\tau}) \end{aligned} \tag{8.15}$$

The isochrones cover the considered area in the state space. This fact is deduced from the observation that $l(\tau, u_m)$ grows monotonically with τ and that $k(\tau)$ is bounded. The time τ is uniquely determined by the state (S, E) and thus defines a function $\tau(S, E)$. This fact is due to the uniqueness of the solution of (8.3) for a given constant control signal.

Now consider a point in the state space on larger distance from the line $E = E_r$. Study the motion (dS_x, dE_x) of the state during the time dt using a control signal x such that $0 \leq x \leq u_m$. The change $d\tau$ of the remaining time τ for which $u(t) = u_m$ is used, is

$$d\tau = \frac{\partial \tau}{\partial S} dS_x + \frac{\partial \tau}{\partial E} dE_x \tag{8.16}$$

If the following condition is satisfied it is worthwhile to change the control signal

$$d\tau + dt < 0 \tag{8.17}$$

The function $\tau(S, E)$ is only implicitly defined by the isochrones (8.15)

$$f\big(\tau(S, E), S, E\big) = E + k(\tau)S - l(\tau, u_m) = 0 \tag{8.18}$$

However, the partial derivatives can be determined by implicit differentiation

$$\begin{aligned} \frac{\partial \tau}{\partial S} &= -\frac{\frac{\partial f}{S}}{\frac{\partial f}{\partial \tau}} = -\frac{k}{\frac{\partial k}{\partial \tau}S - \frac{\partial l}{\partial \tau}} \\ \frac{\partial \tau}{\partial E} &= -\frac{\frac{\partial f}{E}}{\frac{\partial f}{\partial \tau}} = -\frac{1}{\frac{\partial k}{\partial \tau}S - \frac{\partial l}{\partial \tau}} \end{aligned} \tag{8.19}$$

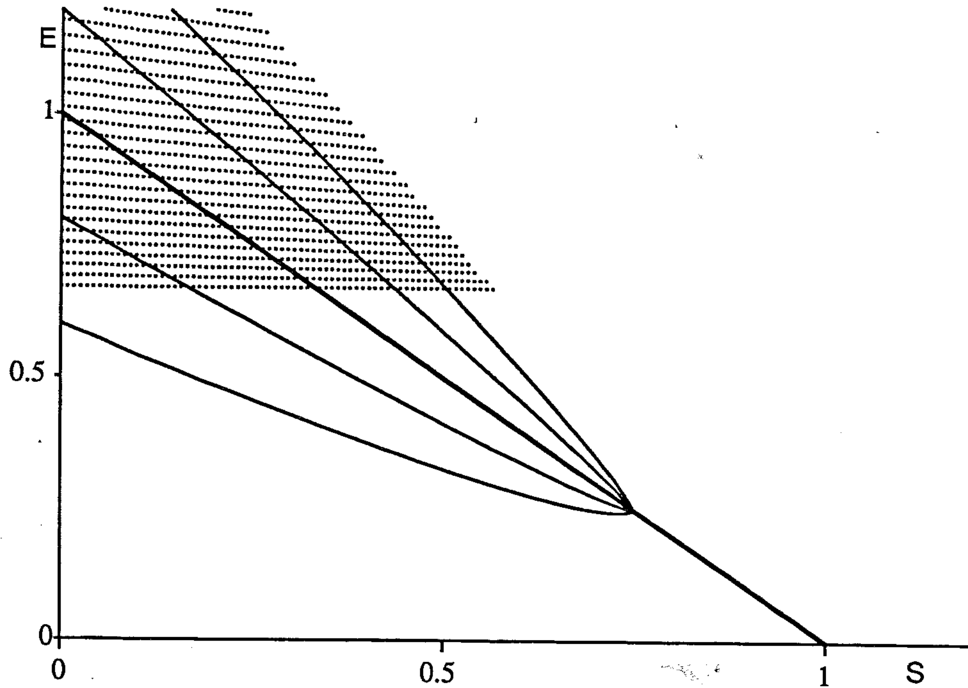


Figure 8.8 Trajectories for $u(t) = u_m$, solid lines, and corresponding isochrones, dotted lines.

and inserted in (8.6) gives

$$d\tau = \frac{k \cdot \big(-S + (1-S)x\big) + 1 \cdot (S - Ex)}{\frac{\partial k}{\partial \tau} S - \frac{\partial l}{\partial \tau}} dt \tag{8.20}$$

thus

$$d\tau + dt = \frac{-Ax + B}{C} dt \tag{8.21}$$

where

$$A = -(1 - e^{-\tau})(1 - S) - E > 0$$
$$B = -(1 - E_r - \frac{u_m}{1 + u_m}) u_m e^{\tau u_m} + \frac{u_m}{1 + u_m} e^{-\tau} > 0$$
$$C = -(S - \frac{u_m}{1 + u_m}) e^{-\tau} - (1 - E_r - \frac{u_m}{1 + u_m}) u_m e^{\tau u_m} > 0$$

The sign of each term is easily determined using the expressions for the stationary points (8.4). The result (8.21) is easily interpreted. For $x = 0$, $d\tau + dt > 0$ and the control variable should not be changed according to (8.7). Further, when x increases $d\tau + dt$ decreases and for $x = u_m$ it is expected that $d\tau + dt = 0$. This is easily checked. The nominator is rearranged for $x = u_m$.

$$Au_m - B = \big(E + k(\tau)S - l(\tau, u_m)\big) u_m = 0$$

Note that the nominator contains a factor that is zero from the definition of the isochrones in (8.15).

Consider the area $E_r - E_r S < E < E_r$, $0 < S$. For states infinitesimally close to the line $E = E_r$

$$\frac{dE}{dt} = S - Eu > 0, \quad u = 0$$

Consider the trajectories in the area for constant control signal $u(t) = 0$. The time to reach $E = E_r$ from any point in this area of the state spaces is denoted $\sigma(S, E)$

$$\sigma(S,E) = -\ln(1 - \frac{E_r - E}{S}) \tag{8.22}$$

Simple calculations reveal that the corresponding isochrones are lines.

$$\begin{aligned} E &= -k(\sigma)S + l \\ k(\sigma) &= 1 - e^{-\sigma} \\ l &= E_r \end{aligned} \tag{8.23}$$

The isochrones cover the area $E_r - S < E < E_r$, $0 < S$ which is clear from the fact that $0 < k < 1$ and that $l = E_r$, thus they cover a larger area than the area under investigation. The same procedure as for the isochrones in the previous area gives

$$\begin{aligned} d\sigma + dt &= -\frac{(1 - e^{-\sigma})(1 - S) - E}{e^{-\sigma} S} x dt \\ &= -\frac{E_r - SE_r - E}{S(S + E - E_r)} \end{aligned} \tag{8.24}$$

The second step is obtained using (8.22). The denominator is positive in the area of interest. The nominator is also positive but changes sign for states below the line

$$E = E_r - SE_r \tag{8.25}$$

The line (8.25) thus defines a switch curve in the state space for the control variable. Above the switch curve the control variable should be zero, and below it should take maximal value. It remains to show that there are no more switch curves below.

Consider the area $0 \leq E \leq E_r - E_r S$, $0 \leq S$. It was previously shown (8.24), that infinitesimally close to the switch-curve (8.25) the control variable should be chosen as large as possible. Therefore consider trajectories with $u(t) = u_m$ in the current part of the phase plane. Calculations show that the isochrones become lines also in this case.

$$\begin{aligned} E &= -k(\tau)S + l(\tau, u_m) \\ k(\tau) &= 1 - (1 - E_r)e^{-\tau} \\ l(\tau, u_m) &= 1 - \frac{1 - E_r}{1 + u_m}(e^{u_m \tau} + u_m e^{-\tau}) \end{aligned} \tag{8.26}$$

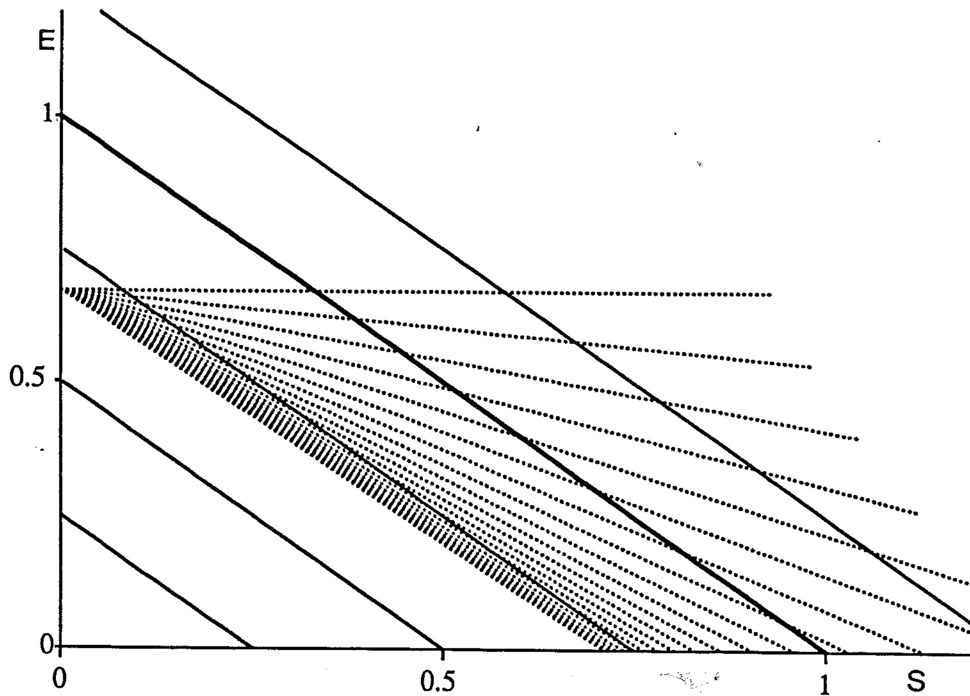


Figure 8.9 Trajectories for $u(t) = 0$, solid lines and corresponding isochrones, dotted lines.

In order to check for another switch-line the standard procedure is repeated

$$d\tau + dt = \frac{-Ax + B}{C} dt \tag{8.27}$$

where

$$\begin{aligned} A &= \big(1 - (1 - E_r)e^{-\tau}\big)(1 - S) - E > 0 \\ B &= \frac{1 - E_r}{1 + u_m}(u_m e^{u_m \tau} - u_m e^{-\tau}) > 0 \\ C &= (1 - E_r)e^{-\tau} S + \frac{1 - E_r}{1 + u_m}(u_m e^{u_m \tau} - u_m e^{-\tau}) > 0 \end{aligned} \tag{8.28}$$

The sign of the coefficient $A(\tau)$ is obvious from the observation that $A(0) > 0$ and that $\partial A/\partial \tau > 0$. The sign of B and C follows immediately. For $x = u_m$ the nominator of (8.27) becomes zero.

$$-Au_m + B = -u_m\big(-E - k(\tau)S + l(\tau, u_m)\big) = 0$$

The result (8.27) implies that for $x < u_m$, $d\tau + dt > 0$ and therefore the control variable should remain $u(t) = u_m$ throughout the examined area. □

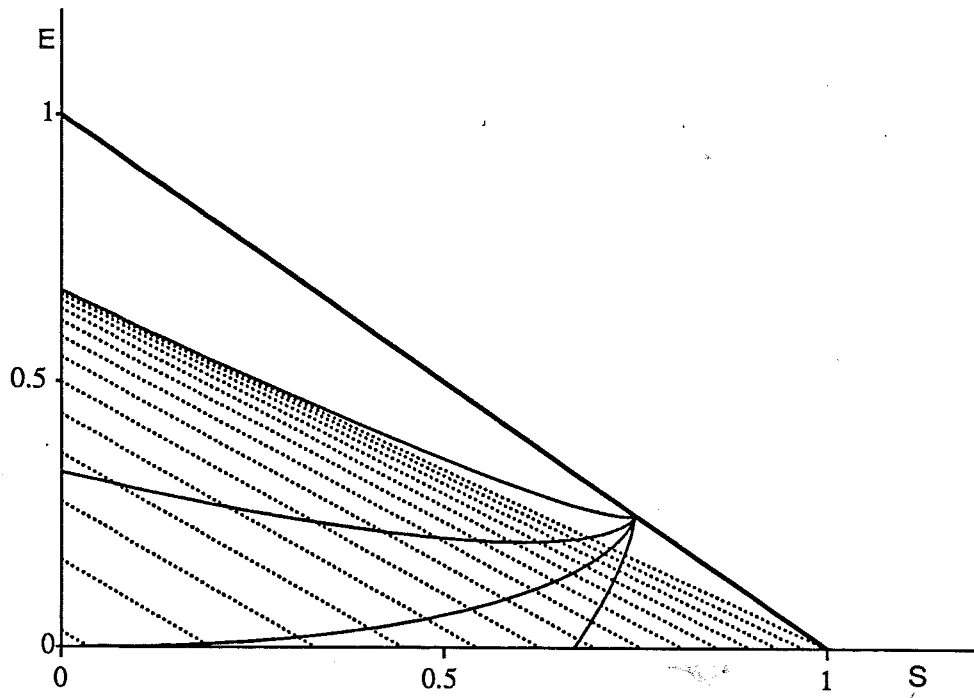


Figure 8.10 Trajectories for $u(t) = u_m$, solid lines, and corresponding isochrones, dotted lines.

8.5 Remarks on controllability concepts and control design

Consider the possibilities to control the process as viewed in the x-coordinates (8.9). From $x_2 = 0$, i. e. the equilibrium line (8.3), the accessible part of the state space is confined to this line. For $x_2 \neq 0$ the whole state space is potentially accessible, except that the equilibrium line cannot be reached in a finite time with a bounded control signal. Thus, the equilibrium line separates the state space into two halves where the state can be controlled. The restriction to a non-negative control variable, also limits the possibilities to control the state. In fact it can only move towards the equilibrium line. However, the rate of this movement can be manipulated by the control variable.

What insight into the control problem can be gained by applying controllability concepts to this non-linear process? For non-linear systems such general questions are much more difficult to answer than for linear systems. Differential geometric concepts have been introduced to discuss properties of non-linear systems and control (Isidori, 1985). It is interesting to see, what insight can be obtained for our process by applying such controllability concepts, and then compare with the insight obtained by inspection of the equations and by the time optimal control formulation. Exact linearization is a general method for a certain class of non-linear plants, that has emerged from the differential geometry school. It is natural to investigate if it could be used here. The results of this section are rather negative but may stimulate further research.

Controllability concepts for linear systems

The process model is linearized around the trajectory $x_1^\circ(t)$, $x_2^\circ(t)$ for a constant control signal u°. The controllability is investigated for a small disturbance around the trajectory given by (8.11). Let $\Delta x = x(t) - x^\circ(t)$ and $\Delta u = u(t) - u^\circ$, then

$$\Delta \dot{x} = \begin{pmatrix} -(1+u^\circ) & 0 \\ 0 & -u^\circ \end{pmatrix} \Delta x + \begin{pmatrix} 1 - x_1^\circ(t) \\ x_2^\circ(t) \end{pmatrix} \Delta u \tag{8.29}$$

The controllability grammian for this linear time-varying system is

$$P(t_2, t_1) = \int_{t_1}^{t_2} \Phi(t_2, \sigma) B(\sigma) B^T(\sigma) \Phi^T(t_2, \sigma) d\sigma \tag{8.30}$$

where

$$\Phi(t_2, \sigma) B(\sigma) = \begin{pmatrix} h(\sigma) \\ x_2(t_0) \end{pmatrix} e^{-u^\circ(t_2 - t_0)} \tag{8.31}$$

and

$$h(\sigma) = e^{-(t_2 - t_0)} \Big(\frac{1}{1+u^\circ} e^{(1+u^\circ)(\sigma - t_0)} + \frac{u^\circ}{1+u^\circ} - x_1(t_0) \Big) \tag{8.32}$$

The controllability grammian has full rank provided

$$\det P(t_2,t_1) = \det \int_{t_1}^{t_2} \begin{pmatrix} h(\sigma) \\ x_2(t_0) \end{pmatrix} \begin{pmatrix} h(\sigma) & x_2(t_0) \end{pmatrix} e^{-2u^\circ(t_2-t_0)} d\sigma =$$

$$= \Big((t_2-t_1)\int_{t_1}^{t_2} h^2(\sigma)d\sigma - \big(\int_{t_1}^{t_2} h(\sigma)d\sigma\big)^2\Big) x_2^2(t_0)e^{-2u^\circ(t_2-t_0)} \neq 0 \tag{8.33}$$

Application of the Cauchy-Schwarz inequality implies that the two integrals cancel, if and only if $h(\sigma)$ is constant. From (8.32) it is evident that $h(\sigma)$ increases with σ. Thus, the grammian is singular if and only if $x_2(t_0) = 0$.

The result that controllability is lost if and only if $x_2(t_0) = 0$ means that a small disturbance Δx from the trajectory $x^\circ(t)$ can be eliminated in a finite time using appropriate variations Δu around the given stationary value u°.

Differential geometric controllability concepts

In the differential geometric language, our process can be described as an interaction between a drift field f and a control field g

$$\dot{x} = f(x) + g(x)u(t) = \begin{pmatrix} -x_1 \\ 0 \end{pmatrix} + \begin{pmatrix} 1-x_1 \\ -x_2 \end{pmatrix} u(t) \tag{8.34}$$

In the previous section it was shown that the first order controllability was lost on the line $x_2 = 0$. Higher order controllability can be analysed with the aid of Lie brackets (Isidori, 1985). A necessary condition for controllability is that g, f, $[f,g]$, $[f,[f,g]]$,... etc., span the state space. Simple calculations gives

$$[f,g] = \frac{dg}{dx}f - \frac{df}{dx}g$$

$$= \begin{pmatrix} -1 & 0 \\ 0 & -1 \end{pmatrix}\begin{pmatrix} -x_1 \\ 0 \end{pmatrix} - \begin{pmatrix} -1 & 0 \\ 0 & 0 \end{pmatrix}\begin{pmatrix} 1-x_1 \\ -x_2 \end{pmatrix} = \begin{pmatrix} 1 \\ 0 \end{pmatrix} \tag{8.35}$$

and further bracketing $[f,[f,g]], [f,[f,[f,g]]], \ldots [g,[f,g]] \ldots$ etc., gives no new directions. Thus, the condition for controllability becomes

$$\det\begin{pmatrix} g(x), [f,g](x) \end{pmatrix} = \det\begin{pmatrix} 1-x_1 & 1 \\ -x_2 & 0 \end{pmatrix} = x_2 \neq 0 \tag{8.36}$$

This shows that there is no higher order controllability on the line $x_2 = 0$. Further, outside the line $x_2 = 0$ the system is controllable, but this was already evident from the previous section.

Exact linearization

The method of exact linearization is a general approach to non-linear control problems. For systems of the form (8.34) there are general results (Hunt *et al*, 1983). The idea is to transform the non-linear system to a linear system by means of a combination of a state space transformation and a feedback control law. Simple calculations show that the following transformation of the state space

$$
\begin{aligned}
z_1 &= \phi_1(x_1, x_2) = -\frac{x_1 x_2}{(1-x_1)^2} \\
z_2 &= \phi_2(x_1, x_2) = \frac{x_2}{(1-x_1)}
\end{aligned}
\tag{8.37a}
$$

and the control variable

$$
u = \psi(x_1, x_2, v) = \frac{1}{\nabla\phi_1 \cdot g} v - \frac{\nabla\phi_1 \cdot f}{\nabla\phi_1 \cdot g} = -\frac{(1-x_1)^2}{x_2} v + \frac{x_1(1+x_1)}{1-x_1} \tag{8.37b}
$$

brings the system to the Brunovsky form

$$
\dot{z} = \begin{pmatrix} 0 & 0 \\ 1 & 0 \end{pmatrix} z + \begin{pmatrix} 1 \\ 0 \end{pmatrix} v(t) \tag{8.38}
$$

Thus, the system behaves as a double integrator between the new control variable $v(t)$ and the transformed state variables $z(t)$. Control design could now be done in these variables. However, note that this design methodology relies on the assumption that good control of the state $z(t)$ implies a nice behaviour of the physical state $x(t)$.

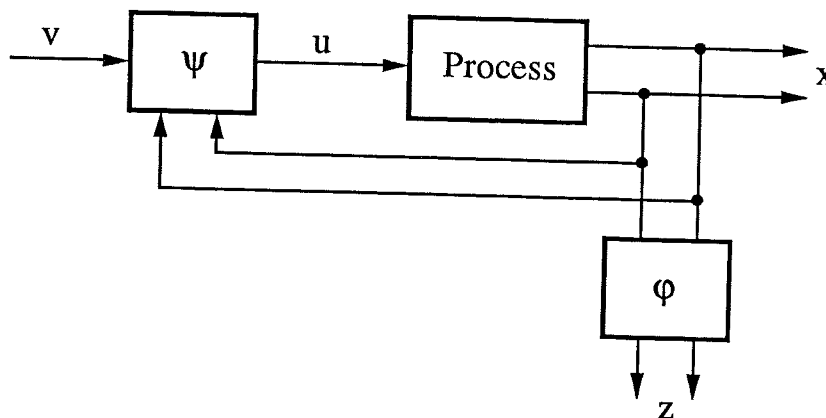


Figure 8.11 Illustration of the exact linearization transformation.

Inspection of the non-linear coordinate transformation (8.37) shows that it is singular. The stationary points $x_2 = 0$ are mapped into a single point in the z-plane, the origin. This means that not only is information about different

stationary points lost in the new coordinates, but also that the neighbourhood of the stationary points are strongly deformed.

The implication of the singularity of the coordinate transformation (8.37) is that feedback control based on the z-coordinates is of little value. An additional difficulty is that the control law (8.37b) will be singular if for instance linear state feedback from z is used.

The roots of these singularities can be traced back to the controllability condition. The fact that the dimension of the controllability space shrinks to one on the line $x_2 = 0$ implies, of course, that no non-singular transformation can bring the system to a controllable form (8.38). For a second order system of the form (8.34) it holds that

$$\det \Big(g(x), [f, g](x) \Big) = 0 \quad \Rightarrow \quad \nabla \phi_1(x) \cdot g(x) = 0 \tag{8.39}$$

where $\phi_1(x)$ is part of the transformation (8.37) that brings the system to the Brunovsky form.

The fact that the control variable is non-negative further complicates application of exact linearization. It is not clear how this limitation should be accounted for.

8.6 Discussion

In this chapter was discussed a non-linear control problem that originated from control of the ethanol concentration in a fermentation process in a continuous tank reactor. Design of the control system based on linear theory was found adequate for set-point control and for elimination of small disturbances. Although set-point changes at different levels call for a slightly different tuning of a linear regulator, reasonable performance can be obtained if a proper choice of parameters is done. Large disturbances and start-up of the process, however, cannot be handled satisfactorily by a linear control law. A different regulator structure is called for. Physical insight gave an idea of how start-up and large disturbances should be handled. Formulation of the control problem as a time optimal problem gave a simple bang-bang control law that for large disturbances, gives control actions in the reversed direction from what a PID control would give.

Analysis showed that a characteristic property of the process is that it is not controllable at the stationary points. The un-controllable mode may be excited by different disturbances. On the other hand, set-point changes do not have any influence on this mode. Under transient conditions in general, the process is controllable and the singularity for stationary points should not be much of a difficulty, after all. However, a design based on the linearized process model around a stationary working point cannot exploit the non-linear property that the un-controllable mode can be manipulated during the transient. Further,

the non-linear method "exact linearization", was not very useful either. The singularity of the process accessability gave rise to a singularity in the non-linear coordinate transformation of the method and made these derived coordinates of little value for feedback control. It is worth considering that in the time-optimal control formulation this somewhat artificial difficulty with controllability never showed up.

Here, only one non-linear aspect of control of continuous fermentation was studied. In Chapter 7 several other control difficulties were touched upon. An important part of the control problem is the limited control authority and this fact may be more pronounced on an industrial scale. Also for such aspects an optimal control formulation could be useful.

9

Conclusion

During the last decade there have been a considerable interest in applications of control to biotechnical processes. In the biotechnical industry as well as in the research laboratories people struggle with the reproducibility of results and the efficiency of processes. Control engineers look for challenging control problems to solve and also as a vehicle to stimulate methodological developments. There is a risk that in such an interdisciplinary project the real problems are forgotten and that the results obtained are interesting for the control engineers only.

This thesis has tried to present an integrated approach to control of biotechnical processes. A laboratory was built up for computer control of fermentation processes and novel on-line measurement techniques were applied. Yeast served as a model organism for study of control of cell growth, and of control of product formation. Simplified dynamical models have been derived and verified by experiments. The underlying biochemistry was elucidated and some questions were raised concerning dynamical aspects of cell metabolism. The obtained process knowledge, in terms of dynamical behaviour, degree of uncertainty and identified sources of disturbances were analysed and used for control design. The proposed regulator structures were compared with conventional PID control. Finally, attempts were made to evaluate the benefits of control in biotechnical terms.

Contributions

The biotechnical contributions of this work are the experimental results and interpretations, and the insight obtained into appropriate regulator structures for the considered processes. The contribution to the field of automatic control is that techniques and concepts for interpretation of data and for control design have been applied and found fruitful to a large extent. There were also examples of drawbacks that are interesting.

An important part of the contribution is the experience with direct measurement of substrates and products. In this way the processes were closely monitored and feedback facilitated reproducible operating conditions. The simultaneous measurement of substrate and product in the continuous fermentation made it possible to confirm a simple model for the tank. For the tube reactor an unexpected fast part of the dynamical response was observed in the outlet product concentration. The ethanol measurement technique was refined in the application to fed-batch yeast production and provided a sensitive indicator of over- and under-feeding of substrate. It was possible to maintain a low and non-zero ethanol concentration throughout a cultivation using feedback control of the substrate dosage. Several cultivations were done with an initial cell concentration of a few grams per litre to a final concentration of 60-70 grams per litre, and the ethanol concentration was kept close to the set-point. These results should be compared with previous work (Dairaku el al, 1983) where difficulties with maintaining the ethanol concentration at a low level were reported, despite the fact that the cultivations were only grown to about half the cell concentration. Thus, it has been shown that ethanol controlled yeast cultivations are possible in the laboratory scale and provides a new tool for microbiological investigations.

The switch of metabolism in yeast has received much interest among microbiologists and biochemists. The development of on-line measurement techniques has facilitated the experimental work and provided possibilities for new insights into cell metabolism (Barford and Hall, 1979; Käppeli and Sonnleitner, 1986). Until recently, the interest has focused around different stationary states of the metabolism. During the last years simple dynamical experiments have been done but the results are often difficult to reproduce and there is certainly lack of microbiological understanding of transient behaviour (Kätterer *et al*, 1986; Sweere *et al*, 1988). The experimental set-up for substrate limited fed-batch cultivation provided a useful tool for making dynamical studies of the yeast metabolism. A series of identification experiments was done at different cell concentrations during a few cultivations. The experiments were done in closed loop to ensure that the feed variations were in the critical range, and thus facilitated reproducibility. Data was fitted to a second order model with good results. The obtained parameters were compared with what could be expected from the underlying biochemistry using the "bottle neck" view (Käppeli and Sonnleitner, 1986) of yeast metabolism. The process gain was found slightly higher than expected, especially at higher cell concentrations. This study showed that the application of closed-loop identification facilitated accurate determination of the dynamics of yeast metabolism and put the current understanding to the test.

The substrate control of fed-batch production of yeast has been studied by several research groups the last decade and there is an emerging industrial interest. In most studies the control objective has been to maintain critical growth rate but different indirect measurement techniques have been used. Thus, the control problems are similar and the designs can be compared to some extent. Previous work has focused on the variations in process dynamics during a cul-

tivation. Parameter scheduling of the regulator has been discussed (Dairaku *et al*, 1983) and several adaptive techniques have been tested (Dekkers *et al*, 1985; Verbruggen *et al*, 1985; Montgomery *et al*, 1985). In this thesis the importance of the variation of dynamics was played down and the ability to track the drastic growth of substrate demand was stressed for design of the control system. The substrate demand was modeled as an exponential load disturbance and an observer was designed. The robustness of the closed loop system was analysed with respect to errors of the disturbance model. An error in the estimate of the initial substrate demand was eliminated and the influence of an error in the growth rate parameter was small. These good properties of the low frequency disturbance rejection were obtained with almost no change of the stability margin of the closed loop system. A moderate phase advance was obtained by derivative action to compensate for the sensor response time. A shift of focus of the problem and a simple application of the internal model principle, thus gave a good performance of a low order regulator with fixed parameters.

Recent developments in non-linear control theory has received much interest and expectation. The method of exact linearization has found several interesting applications. A non-linear control problem was identified in connection with the process for continuous production of ethanol in a tank reactor. Physical insight indicates that the control strategy to compensate for large disturbances are reversed to elimination of small disturbances. A time optimal control problem was formulated and solved using dynamic programming. The obtained control law is simple to understand and provides insight into the problem. The strategy is bang-bang and it is interesting that the switch curve is independent of the maximal control signal. The method of exact linearization turned out to be of less value. The reason is that a singularity turns up in the non-linear coordinate transformation in which the process is linear. The stationary points are mapped into one single point in the new coordinates. The singularity originates from the fact that the process is not controllable on a line in the state space. This line coincide with the line of stationary points. The result of the singularity is not only that information of different stationary states are lost, but also that information about the state is strongly deformed in a neighbourhood of the stationary values. Feedback based on these new coordinates is of little value, and it is impossible to bring the process state back to a certain set-point. Thus, a simple example has been shown that raises questions about fatal deformations of the exact linearization transformation.

The role of simple dynamical models

Biotechnical processes are complex and their dynamical properties are not well known. This is often taken as an argument for introducing adaptive regulators in the control systems. However, in this work two cases were shown where a simple regulator structure would have difficulties. In the fed-batch processes an internal model of the load disturbance is necessary a priori knowledge for good control. In

the continuous tank process the outflow of substance is concentration times flow rate which complicates control for large disturbances. Opposite control actions are called for, compared to the control strategy for small disturbances. This could not be achieved by a regulator with a linear structure. The type of process knowledge mentioned here is straight forward but would facilitate control if it were accounted for.

Increased process knowledge would in general facilitate the control design. It is natural to think in terms of a gradual refinement of the dynamical model, and this could be reflected in the control design. In the fed-batch process the sensor dynamics and delay is easily recognized and the integrator of the reactor dynamics is probable from the bottle-neck view of yeast metabolism. Further insight into the process dynamics demands microbiological knowledge. The value of the gain and how the gain varies for different conditions were discussed out from a stoichiometric model. The dynamics of the switch of metabolism is even more uncertain to derive from the literature. First this latter refinement of the model would be reasonable to leave for an adaptive part of the regulator, to keep the modelling effort at a minimum. In this thesis the modelling work was continued, however, and bounds on the process parameters were found. Analysis showed that a robust design was possible and that further modelling, or adaptation, should rather be concentrated on the growth in the substrate demand.

Even if a robust design will do, it is of interest to have an understanding of how automatic tuning should be done. Here the obtained knowledge of the kind of process uncertainty would be useful. In this way rapid tuning could be facilitated which is crucial for a fed-batch process.

The effort made on modelling cell dynamics might be interesting also for another part of process design, namely design of the reactor. In a large reactor inhomogenities are inevitable and the cell faces rapid changes in the reactor environment (Sweere *et al*, 1988). It is of interest to understand the influence of these fluctuations on overall yield, growth rate etc.

Further research

The dynamical properties of cell cultures is an interesting research area where not much is done. The recent developments of on-line measurement techniques pave the way for new discoveries. Interdisciplinary projects may play a key role, both for developing the experimental techniques and for interpretation of the data. Such research may contribute to basic understanding of cell function and it also has technical relevance. Important variables to control could better be identified and control design facilitated. Reactor design should also benefit from a better understanding of cell dynamics.

The work on dynamics of yeast metabolism is interesting to continue. The experiments show a high degree of reproducibility which is promising for further investigations. Immediate is to understand the deviations between the estimated parameters and the model discussed. One hypothesis is that rapid changes in

the cell metabolism do not affect cell growth and this would explain the observed higher stoichiometric gain. New identification experiments should be done with a more well-defined media and it would be interesting to monitor also other variables in the reactor, for instance the glucose concentration and the oxygen uptake rate. Not only rapid variations in the glucose supply is interesting to study, but also variations in the oxygen- and nitrogen supply. Analysis of the data series from the identification experiment can also be refined. Known structures of the process should be exploited to a larger extent to obtain better parameter estimates.

Study of biotechnical processes may stimulate research on basic concepts in control. A characteristic side of these processes is that they in general have a more uncertain dynamical behaviour than other chemical processes, even though this fact has not stood out in this work. Feedback can be used to reduce the influence of process variations and facilitate reproducible operation. However, quantitative methods to analyse and design for certain reduction of uncertainty are not very well developed. Robust design has become a vivid theoretical research area only recently. This research could benefit from a closer interaction with applications, like for instance control of biotechnical processes.

In the study of the fed-batch process an estimate of the process variations were obtained from a number of cultivations. It was found that the uncertainty in the load disturbance, from the exponential cell growth, was of significance for design of the control system, and the variations in the process dynamics were of secondary importance. The characterization of the load disturbance is a non trivial question. In this thesis a rather crude model was used. A different characterization of the load disturbance would lead to a modification of the control design. The importance of variations in the process dynamics were of secondary importance in the experimental set-up. The influence of these variations on the control system were analysed in the Bode diagram and the crude classical concepts, amplitude and phase margin, were sufficient. However, if for instance the sensor delay time is longer, the control design is more delicate and may call for finer robustness concepts and design methods that exploit knowledge of the uncertainty.

In this thesis a preliminary study was done of control difficulties in continuous ethanol fermentation. Control of the tube reactor appeared to be an interesting control problem and a control design may be of general interest since tube reactors are quite common. In connection with the tank reactor a non-linear control problem was identified and different design methods were applied. The result on time optimal control may be generalized to the tank reactor in general along the lines of (Fjeld *et al*, 1974). For a real design of the control system other performance criteria may be relevant. Also approximate solutions may shed some light on the control problem. Exact linearization was applied to the continuos tank reactor model but the method was found of less value. It would be interesting to further investigate properties of exact linearization transformations and better understand when these are useful.

An important aspect of biotechnical processes is that different reactions are tightly coupled and thus lead to multivariable control problems. In the case of yeast production both substrate addition and aeration are important to control for process economy. The influence of oxygen supply to the cells was briefly discussed in chapter five. It was evident that at low dissolved oxygen concentrations the coupling is tight to how the substrate is utilized and therefore the ethanol concentration in the reactor. A preliminary adaptive multivariable approach to this control problem (Williams *et al*, 1986) show large variations in the control variables and call for a better design. The multivariable nature of biotechnical processes make interpretation of recorded signals difficult. There are also a number of alarm situations that could be detected by analysis of a combination of measured signals. Here expert system techniques may be important to integrate signal analysis with process experience. Another role of expert system ideas may be to integrate sequence control, start-up procedures and ordinary control.

References

AIBA S., S. NAGAI and Y. NISHIZAWA (1976): "Fed-batch cultures of *Saccharomyces cerevisiae*: a perspective of computer control to enhance the productivity in baker's yeast cultivation," *Biotecnol. Bioeng.*, **18**, 1001–1016.

ÅSTRÖM, K. J. and T. HÄGGLUND (1984): "Automatic tuning of simple regulators with specifications on phase and amplitude margins," *Automatica*, **20**, 645–651.

ÅSTRÖM, K. J. and B. WITTENMARK (1980): "Self-tuning regulator based on pole-zero placement," *IEE proceedings part D*, 120–130.

ÅSTRÖM, K. J. and B. WITTENMARK (1984): *Computer Controlled Systems*, Prentice Hall, Englewood Cliffs, New Jersey.

ÅSTRÖM, K. J. and B. WITTENMARK (1988): *Adaptive Control*, Addison-Wesley, Reading, MA.

AXELSSON, J. P. (1985): "Fed-batch cultivation of baker's yeast on glucose media - preliminary experimental results.," Report CODEN: LUTFD2/TFRT-7302, Department of Automatic Control, Lund Institute of Technology, Lund, Sweden.

AXELSSON, J. P. (1987): "Ethanol-controlled fed-batch cultivation of baker's yeast on molasses - experimental experience," Report CODEN: LUTFD2/TFRT-7316, Department of Automatic Control, Lund Institute of Technology, Lund, Sweden.

AXELSSON, J. P. (1988): "Experimental techniques and data treatment for studying the dynamics of ethanol production/consumption in baker's yeast," *Anal. Chim. Acta*, **213**, 151–163.

AXELSSON, J. P. (1988): "Characterizing the substrate control problem of ethanol monitored fed-batch yeast production," in D. Seborg (Ed.): *Adaptive control of chemical processes - ADCHEM'88/IFAC symposium, preprints.*, IFAC, Pergamon Press, pp. 94–99.

AXELSSON, J. P. (1988): "On the role of adaptive controllers in fed-batch yeast production," in D. Seborg: *Adaptive control of chemical processes - AD-CHEM'88/IFAC symposium, preprints.*, IFAC, Pergamon Press, pp. 115–120.

AXELSSON, J. P., P. HAGANDER, C. F. MANDENIUS and B. MATTIASSON (1982): "Computer control of sucrose concentration in a fermentor with continuous flow," in A. Halme (Ed.): *Modelling and Control of Biotechnical Processes - 1st IFAC workshop*, IFAC, Pergamon Press, Helsinki, Finland, pp. 273–280.

AXELSSON, J. P., P. HAGANDER, O. HOLST, C. F. MANDENIUS and B. MATTIASSON (1984): "Control of baker's yeast production based on ethanol measurement," *Third European Congress on Biotechnology, preprint*, Munich, FRG, pp. 629–634.

AXELSSON, J. P. and K. HEDLUND (1986): "Pre-sampling filter for a biotechnical lab," Report CODEN: LUTFD2/TFRT-7311, Department of Automatic Control, Lund Institute of Technology, Lund, Sweden.

AXELSSON, J. P., C. F. MANDENIUS, O. HOLST, P. HAGANDER and B. MATTIASSON (1988): "Experience in using an ethanol sensor to control molasses feed-rates in baker's yeast production," *Bioprocess Engineering*, **3**, 1–9.

BAILEY, J. E. and D. F. OLLIS (1986): *Biochemical Engineering Fundamentals*, McGraw-Hill.

BARFORD, J. P. and R. J. HALL (1979): "Investigation of the significance of a carbon and redox balance to the measurement of the gaseous metabolism of *Saccharomyces cerevisiae*," *Biotechnol. Bioeng.*, **21**, 609–626.

BASTIN, G. and D. DOCHAIN (1986): "On-line estimation of microbial specific growth rates," *Automatica*, **22**, 705–709.

BELLGARDT, K. H. (1984): "Modellbildung des Wachstums von *Saccharomyces cerevisiae* in Rührkesselreaktoren," Ph. D. thesis, Universität Hannover, FRG.

BELLMAN, R. (1961): *Adaptive Control Processes: A Guided Tour*, Princeton University Press, Princeton, New Jersey.

BENGTSSON, G. (1977): "Output regulation and internal models - a frequency domain approach," *Automatica*, **13**, 333-345.

BERRY, D. (1982): *The Biology of Yeast*, Edward Arnold Limited.

BROCKETT, R. (1970): *Finite Dimensional Linear Systems*, John Wiley and Sons.

BRUINENBERG, P. M. J. P. VAN DIJKEN and W. A. SCHEFFERS (1983): "A theoretical analysis of NADPH production and consumption in yeasts," *J. Gen. Microbiol.*, **129**, 953–964.

CHANCE, B., A. K. GHOSH, E. K. PYE and B. HESS (1973): *Biological and Biochemical Oscillators*, Academic Press, New York and London.

COONEY, C. L., H. Y. WANG and D. I. C. WANG (1977): "Computer-aided material balancing for prediction of fermentation parameters," *Biotechnol. Bioeng.*, **19**, 55–67.

CRABTREE, H. G. (1929): "Observations on the carbohydrate metabolism of tumours," *Biochem. J.*, **23**, 536–545.

DAIRAKU, K., Y. YAMASAKI, K. KUKI, S. SHIOYA AND T. TAKAMATSU (1981): "Maximum production in a baker's yeast fed-batch culture by tubing method.," *Biotecnol. Bioeng.*, **23**, 2069–2081.

DAIRAKU, K., Y. YAMASAKI, H. MORIKAWA, S. SHIOYA and T. TAKAMATSU (1982): "Experimental study of time-optimal control in fed-batch culture of baker's yeast," *J. Ferment. Technol.*, **60**, 67–75.

DAIRAKU, K., E. IZUMOTO, H. MORIKAWA, S. SHIOYA and T. TAKAMATSU (1983): "An advanced micro-computer coupled control system in a baker's yeast fed-batch culture using a tubing method," *J. Ferment. Technol.*, **61**, 189–196.

DANIELSSON, B., B. MATTIASON and K. MOSBACH (1981): "Enzyme thermistor devices and their analytical applications," *Applied Biochemistry and Bioengineering*, Academic Press, pp. 97–143.

DE DEKEN, R. H. (1966): "The Crabtree effect. A regulatory system in yeast," *J. Gen. Microbiol.*, **44**, 149–156.

DEKKERS, R. M. (1982): "State estimation of a fed-batch baker's yeast fermentation," in A. Halme: *Modelling and Control of Biotechnical Processes - 1st IFAC workshop*, IFAC, Pergamon Press, Helsinki, Finland, pp. 201–211.

DEKKERS, R. M. and M. VOETTER (1985): "Adaptive control of fed-batch baker's yeast fermentation," in A. Johnson: *Modelling and control of biotechnological processes - 1st IFAC symposium, preprints.*, IFAC, Pergamon Press, pp. 73–80.

DEMAIN, A. and N. SOLOMON (1981): *Scientific American*, September, *Industrial Microbiology*.

ELMQVIST, H., and S. E. MATTSON (1982): "Implementation of real-time facilities in PASCAL," *SOCOCO 3rd IFAC/IFIP Symposium on software for computer control*, Madrid, Spain.

ELMQVIST, H., K. J. ÅSTRÖM and T. SCHÖNTHAL (1986): *Simnon. User's Guide for MS-DOS Computers*, Department of Automatic Control, Lund Institute of Technology, Lund, Sweden.

FJELD, M., O. A. ASBJÖRNSEN and K. J. ÅSTRÖM (1974): "Reaction invariants and their importance in the analysis of eigenvectors, state observability and controllability of the continuous stirred tank reactor," *Chemical Engineering Science*, **29**, 1917–1926.

FUKUDA, H., T. SHIOTANI, W. OKADA and H. MORIKAWA (1978): "A new culture method of baker's yeast to remove growth inhibitory substances," *J. Ferment. Technol.*, **56**, 354–360.

FUKUDA, H., T. SHIOTANI, W. OKADA and H. MORIKAWA (1978): "A mathematical model of the growth of baker's yeast subject to product inhibition," *J. Ferment. Technol.*, **56**, 361–368.

GHOSE, T. E. and R. D. TYAGI (1979): "Rapid ethanol formation of cellulose hydrolysate. Product and substrate inhibition and optimization of fermentor design.," *Biotechnol. Bioeng.*, **21**, 1401–1420.

GUTMAN, P. O. (1981): "Stabilizing controllers for bilinear systems," *IEEE Trans. Automatatic Control*, **AC-26**, 917–922.

HANG, C. C. and K. J. ÅSTRÖM (1987): "Practical aspects of PID auto-tuners based on relay feedback," Report CODEN: LUTFD2/TFRT-7370, Department of Automatic Control, Lund Institute of Technology, Lund, Sweden.

HEINZLE, E., I. J. DUNN, K. FURUKAWA and R. D. TANNER (1982): "Modelling of sustained oscillations observed in continuous culture of *Saccharomyces cerevisiae*," in A. Halme (Ed.): *Modelling and control of biotechnical processes*, IFAC, Pergamon Press, pp. 57–65.

HEINZLE, E., J. MOES and I. J. DUNN (1985): "The influence of cyclic glucose feeding on a continuous baker's yeast culture," *Biotechnology Letters*, **7**, 235–240.

HILL, F. F. and J. THOMMEL (1982): "Continuous measurement of the ammonium concentration during the propagation of baker's yeast," *Process Biochemistry*, **Sept**, 16–18.

HOLST, O., H. HÅKANSON, A. MIYABAYASHI and B. MATTIASSON (1988): "Monitoring of glucose in fermentation processes using a commercial glucose analyser," *Appl. Microbiol. Biotechnol.*, **28**, 32–36.

HUNT, L. R., R. SU and G. MEYER (1983): "Global transformation of nonlinear Systems," *IEEE Trans. Automatic Control*, **AC-28**, 24–31.

ISIDORI, A. (1985): *Nonlinear Control Systems*, Springer Verlag.

JOHNSON, M. J.,J. BORKOWSKI and C. ENGBLOM (1964): "Steam sterilizable probes for dissolved oxygen measurement," *Biotechnol. Bioeng.*, **6**, 457–468.

KAILATH, T. (1980): *Linear Systems*, Prentice Hall.

Käppeli, O. and B. Sonnleitner (1986): "Regulation of sugar metabolism in *Saccharomyces*-type yeast: experimental and conceptual considerations.," *Critical Reviews in Biotechnology.*, **4**, 299–325.

Kätterer, L., H, Allemann, O. Käppeli and A. Fiechter (1986): "Transient responses of continuously growing yeast cultures to dilution rate shifts: a sensitive means to analyze biology and the performance of equipment," *Biotechnol. Bioeng.*, **28**, 146–150.

Kierstan, M. and C. Bucke (1977): "The immobilization of microbial cells, subcellular organelles and enzymes in calcium alginate gels," *Biotechnol. Bioeng.*, **19**, 387–397.

Kobayashi, T.,T. Yano, H. Mori and S. Shimizu (1979): "Cultivations of microorganisms with DO-stat and a silicone tubing sensor," *Biotechnol. Bioeng. Symposium*, **9**, 73–83.

Krouwel, P. G. (1982): "Immobilized Cells for Continuous Solvent Production," Ph. D. thesis, Technical University Delft, The Netherlands.

Larsson, M., V. Arasaratnam and B. Mattiasson (1988): "Effect of poly ethylene glycol on *Saccharomycs cerevisiae* with respect to growth and ethanol production," *J. Microbiol. Biotechnol.*, **3**, 22–29.

Lee, K. J. and P. L. Rogers (1983): "The fermentation kinetics of ethanol production by *Zymomonas mobilis*," *Chem. Eng. J.*, **27**, 31–38.

Lievense, J. C. (1984): "An Investigation of the Aerobic, Glucose-Limited Growth and Dynamics of *Saccharomyces cerevisiae*," Ph. D. thesis, Purdue University, USA.

Lilja, M. (1988): "Least squares fitting to a rational transfer function with time delay," *Preprints Control '88, IEE, Oxford.*

Ljung, L (1986): *System Identification Toolbox - for use with MATLAB, user's guide*, The MathWorks, Inc., Sherborn, Mass., USA.

Ljung, L (1987): *System Identification*, Prentice Hall.

Maiorella, B. L., H. W. Blanch and R. C. Wilke (1983): "By-product inhibition effects on ethanolic fermentation by *Saccharomyces cerevisiae*," *Biotechnol. Bioeng.*, **25**, 103–121.

Maiorella, B. L., H. W. Blanch and R. C. Wilke (1984): "Feed component inhibition in ethanolic fermentation by *Saccharomyces cerevisiae*," *Biotechnol. Bioeng.*, **26**, 1155–1166.

Mandenius, C. F. (1987): "Membrane gas sensors for fermentation monitoring," *J. Ferment. Technol.*, **65**, 723–729.

MANDENIUS, C. F. and B. MATTIASSON (1983): "Improved membrane gas sensor systems for on-line analysis of ethanol and other volatile organic compounds in fermentation media," *Eur. J. Appl Microbiol Biotechnol*, **18**, 197–200.

MANDENIUS, C. F., B. MATTIASSON, J. P. AXELSSON and P. HAGANDER (1987): "Control of ethanol fermentation carried out with alginate entrapped *Saccharomyces cerevisiae*," *Biotechnol. Bioeng.*, **29**, 941–949.

MATTIASSON, B., C. F. MANDENIUS, J. P. AXELSSON, B. DANIELSSON and P. HAGANDER (1983): "Computer control of fermentations with biosensors," *Ann. NY Acad. Sci.*, pp. 193–196.

MCGILVERY, R. and G. GOLDSTEIN (1983): *Biochemistry - A Functional Approach*, Saunders Company.

MEYENBURG, H. K. (1969): "Katabolit-Repression und der Sprossungszyklus von *Saccharomyces cerevisiae*," Ph. D. thesis Nr. 4279, ETH, Zürich, Switzerland.

MEYER, C. and W. BEYLER (1984): "Control strategies for continuous bioprocesses based on biological activities," *Biotecnol. Bioeng.*, **26**, 916–925.

MONOD, J. (1950): "La technique de culture continue: théorie et applications," *Annls. Inst. Pasteur Lille*, **79**, 370–390.

MONTGOMERY, P. A., D. WILLIAMS and B. H. SWANICK (1985): "Control of a fermentation process by an on-line adaptive technique," in A. Johnson: *Modelling and control of biotechnological processes - 1st IFAC symposium, preprints.*, IFAC, Pergamon Press, pp. 81–89.

NAJASHIMA, M., M. AZUMA, S. NAGUCHI, K. INUZUKA and H. SAMEJIMA (1984): "Continuous ethanol fermentation using immobilized yeast cells," *Biotechnol. Bioeng.*, **26**, 992–997.

NÚÑEZ, M., J. and J. M. LEMA (1987): "Cell immobilization: Application to alcohol production," *Enzyme Microb. Technol.*, **9**, 642–651.

OKADA, W., H. FUKUDA and H. MORIKAWA (1981): "Kinetic expression of ethanol production rate and ethanol consumption rate in baker's yeast cultivation," *J. Ferment. Technol.*, **59**, 103–109.

OURA, E. (1972): "The effect of aeration on the growth energetics and biochemical composition of baker's yeast," Ph. D thesis, Helsinki, Finland.

PARULEKAR, S. J., G. B. SEMONES, M. J. ROLF, J. C. LIEVENSE and H. C. LIM (1986): "Induction and elimination of oscillations in continuous cultures of *Saccharomyces cerevisiae*," *Biotechnol. Bioeng.*, **28**, 700–710.

PASTEUR, L. (1861): "Influence de l'oxygéne sur le développement de la levûre et la fermentation alcoolique," *Bulletin de la société chimique de France*, 79–80.

PIRT, S. J. (1975): *Principles of Microbe and Cell Cultivation*, Blackwell Scientific Publications, London.

PONS, M.-N., A. RAJAB and J. M. ENGASSER (1986): "Influence of acetate on growth kinetics and production control of it Saccharomyces cerevisiae," *Appl. Microbiol. Biotechnol.*, **24**, 193–198.

PONS, M.-N. and J. M. ENGASSER (1988): "Monitoring of alcoholic fed-batch cultures by gas chromatography via a gas-permeable membrane," *Anal. Chim. Acta*, **213**, 231–236.

PRO-MATLAB (1987): *User's guide*, The MathWorks, Inc., Sherborn, Mass., USA.

PUNGOR, E. JR., E. J. SCHAEFER, C. L. COONEY and J. C. WEAVER (1983): "Direct monitoring of the liquid and gas phases during a fermentation in a computer-mass-spectrometer-fermentor system," *Eur. J. Appl. Microbiol. Biotechnol.*, **18**, 135–140.

RAJAB, A., J.-M. ENGASSER, P. GERMAIN and A. MICLO (1984): "A physiological model of yeast aerobic growth," *Third European Congress on Biotechnology, preprint, Munich, FRG*, pp. 43–48.

RIEGER, M. (1983): "Untersuchung zur Regulation von Glykolyse und Atmung in *Saccharomyces cerevisiae*," Ph. D. thesis Nr. 7264, ETH, Zürich, Switzerland.

RIEGER, M., O. KÄPPELI and A. FIECHTER (1983): "The role of limited respiration in the incomplete oxidation of glucose by *Saccharomyces cerevisiae*," *J. Gen. Microbiol.*, **129**, 653–661.

ROELS, J. A. (1980): "Simple models for the energetics of growth on substrates with different degrees of reduction," *Biotechnol. Bioeng.*, **22**, 33–53.

ROSE, A. H. and J. S. HARRISON (Ed.) (1971): *The Yeasts*, Academic Press.

SCHEPER, T. and K. SCHÜGERL (1986): "Culture fluorescence studies on aerobic continuous cultures of *Saccharomyces cerevisiae*," *Appl. Microbiol. Biotechnol.*, **23**, 440–444.

SCHLYTER, H. (1982): *De gav sig själva. Den helige Franciskus och några sentida efterföljare*, Skeab Verbum, Sverige.

SHAMA, G. (1988): "Developments in bioreactors for fuel ethanol production," *Process Biochemistry*, **October**, 138–145.

SHAPIRO, J. (1988): "Bacteria as multicellular organisms," *Scientific American*, **June**, 62–69.

SHIOYA, S., H. SHIMIZU, M. OGATA and T. TAKAMATSU (1985): "Simulation and experimental studies of the profile control of the specific growth rate in a fed-batch culture," in A. Johnsson (Ed.): *Modelling and control of biotechnological processes*, IFAC, Pergamon Press, pp. 49–54.

SONNLEITNER, B. and O. KÄPPELI (1986): "Growth of *Saccharomyces cerevisiae* is controlled by its limited respiratory capacity: formulation and verification of a hypothesis," *Biotechnol. Bioeng.*, **28**, 927–937.

SONNLEITNER, B., C. STRÄSSLE and A. FIECHTER (1987): "A structured and segregated model: explanation and description of the stable spontanous synchronization of *Saccharomyces cerevisiae* at low dilution rates," in O. M. Neijssel, R. R. van der Meer and K. Ch. A. M. Luyben (Ed.): *Proc. 4th European Congress on Biotechnology*, Elsevier, pp. 76–79.

SONNLEITNER, B. and A. FIECHTER (1988): "High-performance bioreactors: a new generation," *Anal. Chim. Acta*, **213**, 199–205.

STEPHANOPOULOS, G. and K.-Y. SAN (1984): "Studies on on-line bioreactor identification. Part I: Theory," *Biotechnol. Bioeng.*, **26**, 1176–1188.

STEPHANOPOULOS, G., R. GROSZ and K.-Y. SAN (1984): "Studies on on-line bioreactor identification. Part III: Sensitivity problems with respiratory and heat evolution measurements," *Biotechnol. Bioeng.*, **26**, 1198–1208.

STRÄSSLE, C. (1988): "Modell zur Spontansynchronisation von *Saccharomyces cerevisiae*," Ph. D. thesis Nr. 8598, ETH, Zürich, Switzerland.

STRYER, L. (1981): *Biochemistry*, W. H. Freeman and company, San Francisco, USA.

SU, R. (1982): "On the linear equivalents of nonlinear systems," *System and Control letters*, **2**, 48–52.

SWEERE, A. P. J. (1988): "Response of Baker's Yeast to Transient Environmental Conditions Relevant to Large Scale Fermentation Processes," Ph. D. thesis, Technical University Delft, The Netherlands.

SWEERE, A. P. J., Y. A. MATLA, J. ZANDVLIET, K. CH. A. M. LUYBEN and N. W. F. KOSSEN (1988): "Experimental simulation of glucose fluctuations," *Appl. Microbiol. Biotechnol.*, **28**, 109–115.

SWEERE, A. P. J., J. GIESSELBACH, R. BARENDSE, R. DE KRIEGER, G. HONDERED and K. CH. A. M. LUYBEN (1988): "Modelling the dynamic behaviour of *Saccharomyces cerevisiae* and its application in control experiments," *Appl. Microbiol. Biotechnol.*, **28**, 116–127.

TAKAMATSU, T., S. SHIOYA, H. CHIKATANI and K. DAIRAKU (1985): "Comparison of simple population models in a baker's yeast fed-batch culture," *Chem. Eng.*, **40**, 409–507.

VERBRUGGEN, H. B., G. H. B. EELDERINK and P. M. VAN DEN BROECKE, (1985): "Multiloop controlled fed-batch fermentation process using a selftuning controller," in A. Johnsson (Ed.): *Modelling and control of biotechnological processes*, IFAC, Pergamon Press, pp. 91–96.

WALLER, K and P. MÄKILÄ (1981): "Chemical reaction invariants and variants and their use in reactor modelling, simulation, and control," *Ind. Eng. Chem. Process Des. Dev.*, **20**, 1–11.

WALLSTRÖM, L. (1981): "Tillverkning av jäst," *Livsmedelsteknik*, **7**, 326–328.

WANG, H. Y., C. L. COONEY and D. I. C. WANG (1977): "Computer-aided baker's yeast fermentation," *Biotechnol. Bioeng*, **19**, 69–86.

WILLIAMS, D., P. YOUSEFPOUR and E. M. H. WELLINGTON (1986): "On-line adaptive control of a fed-batch fermentation of *Saccharomyces cerevisiae*," *Biotechnol. Bioeng.*, **28**, 631–645.

WOEHRER, W. and M. ROEHR (1981): "Regulatory aspects of baker's yeast metabolism in aerobic fed-batch cultures," *Biotechnol. Bioeng.*, **23**, 567–581.

WU, W. T., K. C. CHEN and H. W. CHIOU (1985): "On-line optimal control for fed-batch culture of baker's yeast production," *Biotechnol. Bioeng.*, **27**, 755–760.

YAMANÉ, T., M. MATSUDA and E. SADA (1981): "Application of porous teflon tubing method to automatic fed-batch culture of microorganisms. I. Mass transfer through porous teflon tubing," *Biotecnol. Bioeng.*, **23**, 2493–2508.

YAMANÉ, T., M. MATSUDA and E. SADA (1981): "Application of porous teflon tubing method to automatic fed-batch culture of microorganisms. II. Automatic constant-value control of fed substrate (ethanol) concentration in semibatch culture of yeast," *Biotecnol. Bioeng.*, **23**, 2509–2524.

YAMANÉ, T. and S. SHIMIZU (1982): "The minimum sized ideal reactor for continuous alcohol fermentation using immobilized microorganism," *Biotecnol. Bioeng.*, **24**, 2731–2737.

ZABRISKIE, D. W. and A. E. HUMPHREY (1978): "Real-time estimation of aerobic batch fermentation biomass concentration by component balancing," *AIChE J.*, **24**, 138–146.

ZUBAY, G. (1983): *Biochemistry*, Addison and Wesley.

A

Experimental set-up

New on-line measurement techniques were used in combination with standard equipment for laboratory scale fermentation. A flexible micro computer system was used for control and data logging.

On-line measurement techniques

Two different techniques were used for on-line measurements of substrates and products in the broth.

The sucrose concentration was measured using an enzyme thermistor (Danielsson *et al*, 1981). This technique is based on a flow-microcalorimeter containing an immobilized enzyme, in this case invertase, which catalyses the exothermic hydrolysis of sucrose. The produced heat is measured with thermistors placed in close proximity to the enzyme. The temperature difference normally measured is of the magnitude of 0.01° C. The sucrose sensor system had a time delay of 6 min and a time constant of 2 min.

The ethanol concentration was measured with a membrane gas sensor (Mandenius and Mattiasson, 1983; Mandenius, 1987). This technique is based on continuous extraction of volatiles over a semi-permeable silicone membrane. Nitrogen was used as carrier gas. The ethanol concentration in the gas phase was then measured by a semiconducter gas sensor (TGS 812, Figaro Engineering Inc, Osaka, Japan). The ethanol detector is also sensitive to other volatile hydrocarbons, e. g. methanol, propanol, acetic aldehyde, but in the experiments performed the only other compound besides ethanol that could interfere would be acetic aldehyde. The ethanol sensor system had a time delay of 2 min and a time constant of 2 min, cf. Figure 5.4. An early version of the sensor system with a time delay of 8 min, was used in control of ethanol fermentation, cf. Figure 7.6.

Computer control system

A PDP 11/03 microcomputer was used for control, monitoring and data logging. Programs were written in Pascal extended with a real-time kernel (Elmqvist and Mattson, 1982). All signals were galvanically isolated and prefiltered using analog and digital technique (Axelsson and Hedlund, 1986). The digital filter was a second order Butterworth filter with a time constant of 60 s. The sampling interval for the regulators was usually chosen to 30 s, both for control of fed-batch cultivation and continuous ethanol fermentation. However, different sampling intervals in the range 24-130 s were tested for regulators with time delay compensation, cf. Figure 7.3-7.6. The pump signal was under certain periods controlled close to the limitations and an anti-windup mechanism for the integrator was important to incorporate in the regulators (Åström and Wittenmark, 1984).

Configuration for fed-batch yeast cultivation

Cultivations were performed in a fermentor (FLC-B-8 Chemoferm AB, Hägersten, Sweden) with a working volume of 6 L. It was equipped with conventional measurement technique. The membrane gas sensor was used in this set-up. The feed rate was controlled using a voltage controlled peristaltic pump (Ismatec mp-4). A silicone rubber tubing with outer/inner diam 3.3/1.3 mm, 2030-969 LKB products AB Sweden. The voltage to feed rate was linear over the whole actual range 0.0-0.4 L/h. The set-up is shown in Figure 4.1.

Cultivation conditions Cultivations were grown under substrate limited conditions using feedback computer control based on measurement of the ethanol signal. Other variables were kept constant using conventional regulators. The temperature was controlled at 30°C. Two molar $NaOH$ was used to keep the pH constant at 5.0. Foam was controlled by addition of the antifoam Glanapon DG 111. Aeration rate was 1.0 volume/volume · min. Dissolved oxygen was measured using a galvanic oxygen electrode (Johnson *et al*, 1964).

Cultivations have been carried out in three stages: growth of inoculum, batch and fed-batch cultivation. The growth of inoculum took 16 h, the batch cultivation 12-14 h, and after a two hour pause the fed-batch cultivation started and continued for about 18 h. One critical stage was the start-up of the fed-batch cultivation. The end of the batch cultivation is clearly indicated by the sudden rise of the dissolved oxygen signal and the level off of the signal from the ethanol sensor. The zero level of the ethanol signal was at this point recalibrated. The culture was starved for two hours, and then it was given a substrate pulse of 20 g of the fed-batch media. In this way the ethanol concentration of the broth rose quickly and came close to the set-point 0.4 g/L, within 45 min. The regulator was then started. The fed-batch cultivation continued for 18 h.

The inoculum was cultivated in shaker flasks for 16 h at 30°C on 50 g/L molasses, 5 g/L NH_3, 5 g/L H_3PO_4 and 2.5 g/L $MgSO_4 \cdot 7H_2O$. The magnesium salt was sterilized separately. The pH was adjusted to 5.5 with $H_2SO_4/NaOH$.

The medium in the batch was composed as follows: 10 g/L molasses, 9.38 g/L NH_3, 8.75 g/L H_3PO_4, 4.75 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L $ZnSO_4 \cdot 7H_2O$, 0.15 mg/L biotin and 4.5 mg/L thiamine. The pH was adjusted to 5.5 with $H_2SO_4/NaOH$. The molasses were diluted 1:1 and centrifuged at 8000 G for 10 min to remove suspended solids. The magnesium salt was sterilized separately and the vitamins were sterile filtered (Scheicher and Schuell, membranfilter 0.45 μm). The stirrer speed during the batch phase was set to 400 rpm.

The fed-batch feed contained 1.69 kg molasses with density 1.41 g/L which was mixed with 1 L H_2O and then centrifuged at 5000 G for 20 min to remove suspended solids. This gives a feed concentration of 0.628 g(molasses)/g(feed) or about 410 g(glucose equivalent)/L(feed). The pH was adjusted to 5.5 with $H_2SO_4/NaOH$.

Analysis Dry weight measurements were taken to determine cell concentration. Samples of the broth were taken from an outlet at the bottom of the fermentor. From each sample two or more dry weight filters (0.45 μm) were prepared. Distilled water was used to wash the filter. Filters were dried for 75 min at a temperature of 105° C and later the weight was measured. In the meantime the filter was kept dry.

The yield calculations in Chapter 4 are based on dry weight measurement of the cell concentration, volume of the broth, and measurement of the amount of feed consumed. The volume was calculated from the known initial volume, amount of substrate and sodium hydroxide fed, and subtraction of the volume of samples taken. The amount of feed consumed was monitored using a load cell on which the substrate vessel was placed.

Configuration for continuous ethanol fermentation

A tank reactor of 5 L and a tube of 1.5 L (length 900 mm; inner diameter 50 mm) was used in combination. The tank had a conical shape that gave favourable mixing conditions of the alginate beads. The tube was sectioned in three compartments, each with passage for liquid and gas between the sections. The flow rate was controlled using a peristaltic pump (Ismatec mp-4) modified for flow rates in the range 0-9 L/h. The liquid was removed from the tank and pumped into the tube using suction pump (Masterflex, Cole-Palmer). The level of the tank was also controlled in this way. The process was operated at room temperature and no additional temperature control was used. The set-up is shown in Figure 7.1.

Immobilization in Ca-alginate gel The alginate gel in the tank and that in the tube reactor was produced according to (Kierstan and Bucke, 1977). Baker's yeast, *Saccharomyces cerevisiae*, from Svenska Jästbolaget AB was used. It was added to an alginate solution so that 1 g of the final gel preparation contained 0.25 g wet yeast cells. The beads for the tank was 2 mm in diameter, and for the tube 3-4 mm.

B

Yeast culture model – a Simnon code

This appendix contains a Simnon code of the yeast model presented in Chapter 3. Simulations can be done on a PC (Elmqvist *et al*, 1986). The model is based on the bottle-neck principle. The main ideas and the numerical values used are taken from (Sonnleitner and Käppeli, 1986). However, in the article by Sonnleitner and Käppeli the uptake rates of glucose and ethanol are described by Monod type dependencies of concentrations while linear relations are used here. The main assumptions of the model can be summarised as follows.

- The substrate concentration drives the uptake rates q_G, q_E and growth rate μ.
- Glucose and ethanol can simultaneously be consumed, but since the respiratory capacity $q_{O_2}^{lim}$ is limited, glucose is consumed preferably.
- The respiratory capacity is constant.
- The cell adjusts immediately to changes in the substrate concentrations.
- The growth rate can be viewed as a linear combination of three terms.
- The cell content remains constant despite variations in growth rate.

The model of the yeast culture also contains the equations for a homogeneous reactor with volume V. The inflow and outflow are denoted F_{in} and F_{out} respectively. The inflow contains a substrate with glucose concentration G_{in} and ethanol concentration E_{in}. This model structure makes it simple to simulate both fed-batch and continuous cultivation. The connecting systems for fed-batch cultivation with a basic dosage scheme are given in Listing B.2 and the connecting system for simulation of chemostat experiments with mixed media are given in Listing B.3.

```
CONTINUOUS SYSTEM culture

INPUT Fin Fout Gin Ein
OUTPUT E RQ
STATE VG VE VX V
DER dVG dVE dVX dV

"Concentrations:
G  = VG/V
E  = VE/V
X  = VX/V
Gm = G/MwG
Em = E/MwE

"Mass balance for the reactor:
dVG = -qG*VX + Gin*Fin - max(G,0)*Fout
dVE =  qE*VX + Ein*Fin - max(E,0)*Fout
dVX =  mu*VX           - max(X,0)*Fout
dV  =            Fin -           Fout

"Growth rate:
mum = YGox*qGox+YGred*qGred+YEox*qEox
mu  = MwX*mum

"Oxidative and oxido-reductive pathways:
qGox  = min(qGm,qGoxlim)
qGred = max(qGm-qGox,0)
qEox  = min(qEoxpot,qEoxlim)

qGoxlim = qO2lim/kog
qEoxlim = max((qO2lim-kog*qGox)/koe,0)

"Actual oxidation rate:
qO2 = kog*qGox+koe*qEox

"Sugar uptake rate:
qGm = qGOm+alfa*(Gm-GOm)
qG  = MwG*qGm

"Ethanol formation/uptake rate:
qEoxpot = gamma*Em
qEm = -qEox+keg*qGred
qE = MwE*qEm
```

```
"Carbondioxide formation rate:
qCO2 = kc1*qGox+kc2*qGred+kc3*qEox

"Respiratory quotient:
RQ = IF qO2>eps THEN qCO2/qO2 ELSE 0

"PARAMETERS

"Initial values:
V       : 4.0       "L        initial volume
VX      : 10        "g        initial cell mass

"Mole weights:
MwG     : 180       "g/mole
MwE     :  46       "g/mole
MwX     :  25       "g/mole             ash content neglected

"Physiological parameters:
qO2lim : 8.0E-3    "mole/g,h
GOm     : 0.55E-3   "mole/L             critical glucose conc
qGOm    : 2.7E-3    "mole/g,h           corresponds to GO=Gcrit
alfa    : 8.5       "1/g,h
gamma   : 1         "1/g,h              an educated guess

kog     : 2.3       "mole(O2)/mole(G)   stoichiometric coeff a
koe     : 1.6       "mole(O2)/mole(E)                  "    k
keg     : 1.9       "mole(E)/mole(G)                   "    j

YGox    : 3.5       "mole(X)/mole(G) oxidatively       "    b
YGred   : 0.36      "mole(X)/mole(G) reductively       "    g
YEox    : 1.32      "mole(X)/mole(E) oxidatively       "    l

kc1     : 2.5       "mole(CO2)/mole(G) oxidatively     "    c
kc2     : 1.89      "mole(CO2)/mole(G) reductively     "    h
kc3     : 0.68      "mole(CO2)/mole(E) oxidatively     "    m

eps     : 1E-4

END
```

Listing B.1 Simnon code for the yeast culture model presented in Chapter 3.

```
CONNECTING SYSTEM fedbatch

TIME t

Gin[culture]  = 400              "g/L
Ein[culture]  = 0                "g/L
Fin[culture]  = Fb               "L/h
Fout[culture] = 0                "L/h

Fb            = F0*exp(mu*t)     "L/h

"Parameters
F0            : 0.01             "L/h
mu            : 0.20             "1/h

END
```

Listing B.2 Connecting system for simulation of a fed-batch cultivation using a basic dosage scheme $F_b(t) = F_0 \exp(\mu t)$.

```
CONNECTING SYSTEM chemostat

TIME t

Gin[culture]  = 15               "g/L
Ein[culture]  = 5                "g/L
Fin[culture]  = V[culture]*D     "L/h
Fout[culture] = V[culture]*D     "L/h

D             = IF t<t1 THEN D1 ELSE D2

"Parameters:
t1            :  5               "h
D1            :  0.15            "1/h
D2            :  0.18            "1/h

END
```

Listing B.3 Connecting system for simulation of a chemostat experiment. The feed contains a mixture of glucose and ethanol and the dilution rate D is changed at $t = 5$ h.