The Application of
Model-based Observer Control
to Bioreactors

Department of Chemical & Process Engineering,
University of Surrey, Guildford, Surrey GU2 5XH, UK.

Dissertation submitted by
SÓNIA MARIA MACHADO ARAÚJO
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Para os meus pais,
Guilhermina e Gastão,
e irmão, TãoZé,
pelo incomensurável apoio e encorajamento.
Bem hajam! *


*To my parents, Guilhermina and Gastão, and brother, TãoZé, for the incommensurable support and encouragement. Thank you! (expression with no direct translation)
Abstract

The application of model-based observer (MBO) control to bioreactors is proposed. In this control strategy, a model is used to infer process information on-line. The model employed in this work was built within the modelling framework CELCYMUS and is used to predict cell age distribution on-line. A copy of the model is used as the process; the control strategy is thus applied to a simulated bioreactor.

The MBO controller encompasses an algorithm to adapt the model and another to control the process. These algorithms are developed and tested separately; batch and repeated batch operation are considered. The model is successfully adapted except when manipulating parameters directly related to the model structure. Process control is achieved by manipulation of a process parameter with respect to cell age distribution; additionally using set point error leads to either the same or worse process performance.

The above algorithms are subsequently integrated into an overall MBO control algorithm. In general, their interaction is minimal, although it results in successful adaptation for the parameters related to model structure. The MBO control algorithm developed is capable of enhancing process performance even when considering, separately or in combination, low sampling frequencies, presence of noise, presence of hidden mismatches, presence of human errors and a wide range of operational conditions.

The objectives of this dissertation have been met. On-line prediction of cell age distribution has proven paramount in controlling a simulated bioreactor. In fact, it was concluded that it would be advantageous to control the process uniquely based on this information, disregarding set point error. The results obtained pre-empt success for the application of MBO control to bioreactors.
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Abbreviations

Ammo ammonia concentration in the medium (kg m⁻³)
Anti antibody concentration in the medium (kg m⁻³)
ANN artificial neural network
ARD acoustic resonance densitometry
CELCYMUS Cell Cycle Model, University of Surrey
Cell viable cell concentration (cell m⁻³)
D death phase of the cell cycle
DCT dilution cycle time (h)
DNA deoxyribonucleic acid
FIA flow injection analysis
G0 quiescent phase of the cell cycle
G1 phase between the M and S phases
G1’ quiescent phase in the model used associated with the initial lag period
G1a first period of the G1 phase
G1b second period of the G1 phase
G2 phase between the S and M phases
Glut glutamine concentration in the medium (kg m⁻³)
GMC generic model control
HF harvest fraction (%)
HPLC high performance liquid chromatography
mm321 mouse-mouse hybridoma, producing IgG antibody to paraquat
MBO model-based observer
M mitosis phase of the cell cycle
NIR near infrared spectroscopy
PID proportional-integral-derivative
PMBC physical-model based control
S DNA synthesis phase of the cell cycle
Nomenclature

a, b normally distributed values
C medium concentration of a particular component (kg m⁻³)
CC intra-cellular concentration of a particular component (kg m⁻³ cell⁻¹)
e error (units as appropriate)
e_u ultimate model error (units as appropriate)
e_ov overshoot of the model error (units as appropriate)
F flow rate (m³ h⁻¹)
F an arbitrary rule of the set defined for a particular phase
G number of transition rules defined for a particular phase
k death rate constant ((m³)¹.⁵ kg⁻¹.⁵ h⁻¹)
K proportional gain (units as appropriate)
K_a model adaptor gain (units as appropriate)
K_c process controller gain (units as appropriate)
m manipulated variable (units as appropriate)
n population density function (cell m⁻³ h⁻¹)
N_p total number of phases
P average net transport rate per cell of a particular component (kg cell⁻¹ h⁻¹)
P_C average net internal production rate per cell of a particular intra-cellular component (kg cell⁻¹ h⁻¹)
r_D cell death rate (cell m⁻³ h⁻¹)
R rate constant for the transport of a particular component into a cell in phase X (units as appropriate)
R_Anti specific antibody production rate (kg h⁻¹ cell⁻¹)
R_Glut specific glutamine uptake rate (kg h⁻¹ cell⁻¹)
S Max maximum amount of glutamine a cell can consume during the G1b phase, before it has to proceed to the S phase (kg cell⁻¹)
SP set point (units as appropriate)
Nomenclature

\( t \) \quad \text{time (h)}

\( T \) \quad \text{duration of a particular phase (h)}

\( t_c \) \quad \text{cross time (h)}

\( t_s \) \quad \text{settling time (h)}

\( u_1, u_2 \) \quad \text{uniformly distributed values}

\( V \) \quad \text{bioreactor working volume (m}^3\text{)}

\( x \) \quad \text{model parameter (units as appropriate)}

\( y \) \quad \text{measured output (units as appropriate)}

\( z \) \quad \text{process parameter (units as appropriate)}

Subscripts

\( a \) \quad \text{model adaptor}

\( b \) \quad \text{bias}

\( c \) \quad \text{process controller}

\( I \) \quad \text{inlet}

\( K \) \quad \text{an arbitrary medium component}

\( L \) \quad \text{an arbitrary intra-cellular component}

\( m \) \quad \text{model}

\( p \) \quad \text{process}

\( \text{par} \) \quad \text{parameter}

\( \text{SP} \) \quad \text{set point}

\( X \) \quad \text{an arbitrary phase}
Greek letters

\( \tau \) biological age (h)
\( \tau_a \) integral action time for the model adaptor (units as appropriate)
\( \tau_c \) integral action time for the process controller (units as appropriate)
\( \tau_D \) derivative action time (units as appropriate)
\( \tau_I \) integral action time (units as appropriate)

Vectors

\( \mathbf{C} \) medium state vector (kg m\(^{-3}\))
\( \mathbf{C}_C \) cytological state vector (kg m\(^{-3}\) cell\(^{-1}\))
\( \mathbf{R}_C \) cytological rate constant vector (units as appropriate)
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Chapter 1

Introduction

The concept of control has accompanied Mankind since the beginning of time in the sense of manipulating events or 'things' in a desired way. Better planned control emerged with the practice of farming in the Neolithic period; the development and diffusion of mathematics, from ancient civilisations to modern times, led to increasingly complex control. Currently, control affects all sectors of the Human society.

In an industrial context, process control emerged from the need to produce what was required, in the way it was required (Stephanopoulos, 1985), in order to ensure productivity and reduce or eliminate operational constraints. Initially, process control was limited to experimental observation and modifying simple instrumentation, such as opening or closing valves, in order to change process behaviour. The development of digital technology and the advent of computer science in the 1970s led to dramatic changes in process control. Computers provided rapid data acquisition, reduction and storage (Wang et al., 1986) and, thus, more efficient process monitoring. Furthermore, they enabled operators to reach faster, more effective decisions and could themselves be programmed to dictate operational policy (Stephanopoulos, 1985).

Despite the progress achieved in the last three decades, industry sometimes seems reluctant to embark on novel control strategies as the balance between the cost of implementing such strategies and the profit gained is not always clear. Much of the progress in control has thus been achieved as a result of academic research and only the end result, if at all, is transferred to the plant. This contributes to control often being viewed in industry as a 'mystic art' (Montague, 1997).

Several control objectives or requirements are now considered when applying process
control in order to force a process to perform acceptably (Montague, 1997). Process control is required not only to enhance productivity and reduce operational constraints, but also to reduce or eliminate the effect of external influences upon the process, which lead to a spread of performance without control action. A consequence of these external disturbances is the variation in product quality, which is undesirable for the customer and, in some cases, in regulatory terms (Montague, 1997). Furthermore, the financial benefits from effective control are also clear. Increasing safety rules and more stringent environmental regulations have become important control requirements in more recent years. Modern, capitalist society has also introduced pressures to industry associated with increased competition and rapidly changing economic conditions (Seborg et al., 1989). These pressures translate into stricter control measures and an increasing need for novel, more efficient control strategies.

The availability of inexpensive hardware and software for monitoring and control has been paramount in achieving the above control requirements. This computational power enabled the development and application of robust control strategies, capable of controlling simple systems more effectively, but also complex systems. These strategies have been applied across a wide range of manufacturing and process engineering areas. The work presented in this dissertation focus on the application to bioreactor operation of a control strategy which has proven successful for chemical engineering processes.

In general, conventional bioreactor control is poor, mainly due to the lack of appropriate sensors and on-line measurement techniques for monitoring and control of key process variables. The difficulty in obtaining that process information is a direct consequence of the complexity of the processes involved, namely their inherent dynamic and non-linear nature, and also of the propagation methods employed (Zeng et al., 1992; Lübbert and Simutis, 1994). The limitations in bioreactor control can be overcome by the development of instrumentation and measurement techniques. However, it has been observed that monitoring of important process information is still a difficult problem and the solutions which have been proposed are, in general,
either unreliable or uneconomical (Locher et al., 1992; Sonnleitner et al., 1992; Zorzetto, 1995; Bogle et al., 1996; Montague, 1997).

Modelling provides a means to estimate process information on-line and continuously. Therefore, it may be used as an alternative to the monitoring of key process variables. However, the use of such an approach for control purposes can only be successful if the models employed are a good representation of the process. For example, simple mathematical descriptions have limited application due to the nature of their inherent simplifying assumptions. In the terminology of bioprocess modelling (Fredrickson and Tsuchiya, 1963), structured, segregated models are required for effective estimation of process information (Fredrickson et al., 1967; Faraday, 1994). These models give a quantitative and qualitative representation of the process (Harder and Roels, 1982), whilst considering the biophase to comprise of individual, heterogeneous entities (Bailey and Ollis, 1986); thus, they are able to distinguish between cell growth and reproduction (Faraday, 1994). The main limitation of using structured, segregated models is the mathematical complexity involved in their development and solving (Fredrickson et al., 1967; Ramkrishna, 1979; Faraday, 1994).

Several workers have attempted to develop structured, segregated models to mimic process behaviour (Fredrickson and Tsuchiya, 1963; Fredrickson et al., 1967; Faraday, 1994), and application of such models for control of biological processes has also been suggested. Further application of models to bioreactor operation has been proposed with the introduction of artificial neural networks and fuzzy logic. Neural network models enable correlating unmeasurable variables to measured data, without any knowledge of the process mechanism, whereas fuzzy models provide a qualitative description of the system. Although attractive, these models do not contribute to the understanding of the complex dynamics of biological processes.

Incorporation in control algorithms of process models, either linear or non-linear, has now wide application in bioreactor operation (Williams et al., 1986; Ramseier et al., 1993; Zeng et al., 1993a; King, 1997). Different variations in the way models are used have been suggested; quite often, these are the consequence of successful control of
chemical reactors. An approach which has been successfully applied to chemical reactors is model-based observer (MBO) control (Jones and Gawthrop, 1992; Gawthrop and Ponton, 1996). The control algorithm employed in this strategy uses process information estimated on-line by a model, but is also capable of updating or correcting that model whenever necessary so that model predictions are useful for control purposes.

In this dissertation, the application of MBO control to bioreactors is considered. The success of this control strategy depends on the existence both of a predictive and robust process model and of an adequate control algorithm. The process model employed was built within the modelling framework CELCYMUS (Faraday, 1994). The specific model used is a structured, segregated model for a hybridoma cell line, and has previously (Faraday, 1994) proven successful in simulating and predicting the growth and production kinetics for this cell line. This model describes the population in terms of cell age and was used to estimate on-line the distribution of cell ages throughout a population. This distribution enables assessing the inoculum state at any time, which directly affects cell population dynamics (Faraday, 1994). It is evident that this information could prove extremely useful for bioreactor control but, currently, there is no reliable or economical method for the on-line monitoring of this parameter. The use of a model to estimate cell age distribution is thus both justifiable and desirable.

The control algorithm developed for the application of the MBO control strategy comprises two algorithms. One control algorithm is employed to enhance process performance, based on measured process information and the cell age distribution inferred by the model. The second algorithm is used to correct any process/model errors, by updating specific model parameters.

The aim of this dissertation is to show that MBO control can be successfully applied to bioreactors, using a model built within CELCYMUS, and that this control strategy will result in greater performance enhancement than conventional control. To achieve this aim, the following objectives were identified for the research work:
to develop and test a control algorithm capable of correcting the model on-line when a model parameter is initially altered;

- to develop and test a control algorithm capable of enhancing process performance using information inferred on-line by a model; and

- to integrate the above algorithms into a MBO control algorithm and investigate whether the overall algorithm is capable of enhancing process performance on-line, whilst correcting the model, whenever necessary, for different culture conditions and propagation methods.

This dissertation includes detailed description of the methodology employed to develop the above algorithms. Furthermore, a detailed analysis of the results obtained when testing the algorithms is also included; these will enable assessing the applicability of MBO control for bioreactor operation.

A copy of the model has been employed instead of a real process. Therefore, MBO control was applied to a simulated bioreactor. This was justified on the basis that there were no previous data or literature on the application of MBO control to bioreactors. Therefore, using a real bioreactor would have been too time consuming and the experiments conducted would have been unrealistically expensive. However, it should be noted that this was only possible due to the robustness of the model used.

Finally, it should be remarked that this research work was a direct consequence of that conducted by Faraday and Kirkby (Faraday, 1994). The incorporation of CELCYMUS, and models built within it, in a MBO control strategy was suggested by those workers as a potential future application of this framework. The work presented in this dissertation will demonstrate the success of that recommendation.
Chapter 2

Literature Survey

2.1 Introduction

As stated in Chapter 1, the application of model-based observer (MBO) control to bioreactors is proposed in this dissertation. This research emerges from the need to develop alternative control strategies for bioreactor operation as conventional strategies, in general, lead to poor control.

The limitations observed in conventional bioreactor control are mainly due to the lack of on-line measurements of important process information and to biological processes being complex and, thus, not yet fully understood. Advances in both monitoring and modelling have contributed enormously to improving bioreactor control and to the development of alternative control strategies. The availability of reliable and inexpensive computational power has played an important role in these achievements. A brief survey of relevant publications will be presented in this chapter in order to identify the trends in current control research, particularly for bioreactor operation. Furthermore, an overview of monitoring and modelling concepts and literature will also be included.

2.2 Monitoring

Process monitoring involves registering the evolution with time of process variables; this information may be used in a multitude of ways, namely for control purposes. However, monitoring will only be useful if it is possible to obtain accurate and precise process data. This can be achieved by employing robust sensors and reliable measurement techniques. Improving the robustness of sensors or constructing new
ones is limited by market size, due to financial incentives (Montague, 1997). Furthermore, using sophisticated instrumentation may not always be advantageous as the cost of installing and running such devices may outbalance the benefits achieved. In addition, sensors in bioreactors cannot interfere with the process or in any way cause contamination. Data quality may also be improved by replacing or improving the measurement technique employed. A brief overview of the types of techniques used in bioreactor operation will be given below.

2.2.1 Measurement techniques for bioreactors

Measurement techniques may be divided into on-line and off-line techniques; comprehensive reviews on their application to bioreactor operation have been presented elsewhere (Wang and Stephanopoulos, 1984; Locher et al., 1992; Sonnleitner et al., 1992; Konstantinov et al., 1994; Montague, 1997).

In off-line techniques, a time lag exists between data acquisition and interpretation; this may give rise to misleading information, which is of little or no use for control purposes (Montague, 1997). On-line measurement results in the elimination of that time lag. However, the information provided may also be misleading if the sampling frequency is too low (Montague, 1997).

There is a great variety of reliable off-line techniques for bioreactor monitoring, namely for determining substrate and product concentrations (Sonnleitner et al., 1992). Furthermore, computer imaging is increasingly being applied to bioreactor monitoring, e.g., for off-line cell counting and sizing of animal cell culture samples (Konstantinov et al., 1994). On-line measurement techniques for bioreactors are more scarce, mainly due to the lack of reliable and economical instrumentation.

In industrial applications, on-line monitoring is usually restricted to the measurement of pH, temperature, pressure or CO₂ and O₂ in the exhaust gas (Agrawal, 1989; Chang and Lim, 1990; Ramseier et al., 1993). In-situ fluorometry (Beyeler et al., 1981;
Bambot et al., 1995) and flow cytometry (Melamed et al., 1979; Hutter et al., 1979; Bailey and Ollis, 1986) have been developed for determining cell properties on-line, or almost, although data reliability and consistency cannot be achieved with these techniques (Bailey and Ollis, 1986).

Some of the off-line techniques can be, and have been, adapted for on-line measurements; the success and feasibility of the adaptation vary from process to process. On-line gas chromatography and high performance liquid chromatography (HPLC) have been used for biomass determination (Sonnleitner et al., 1992) and automated glucose sensors have also been developed (Queinnec et al., 1992). However, these methods require expensive sampling devices and create long measurement time delays. Therefore, on-line measurement of important process state variables, such as cell mass, substrate and product concentrations, remains a difficult problem (Locher et al., 1992), which can only be solved by employing new, reliable measurement techniques or developing adequate, economical instrumentation.

2.2.2 Advances in on-line measurement

The more recent development of on-line monitoring has focused on the utilisation of on-line techniques such as: flow injection analysis, FIA (Filippini et al., 1991), high performance liquid chromatography, HPLC (Van de Merbel et al., 1992), acoustic resonance densitometry, ARD (Konstantinov et al., 1994) and near infrared spectroscopy, NIR (Macaloney et al., 1996; Montague, 1997). However, many probes have a non-linear response and suffer from severe noise problems when used in bioreactors with high aeration rates, with solids in the medium or with protein stabilised foaming (Bogle et al., 1996). Furthermore, time delays, clogging and non-sterility mean that the design of probes is difficult and their active life can be severely diminished (Bogle et al., 1996; Montague, 1997). The cost and complexity of such systems generally make them uneconomic.

Estimation methods can be a useful alternative. They make use of readily available
information, together with some form of mathematical representation of the process, i.e., a model, to deduce on-line a quantity which is difficult, expensive or impossible to measure (Stephanopoulos and San, 1984; Montague, 1997). In addition to providing information otherwise unavailable, on-line estimation yields continuous information, thus eliminating the uncertainty and speculation about the events that take place between consecutive samplings (Stephanopoulos and San, 1984). However, the estimators are usually based on relatively simple models with extensive simplifying assumptions as bioprocesses are generally not completely understood.

Another advance in monitoring is the use of an algorithm to combine the information obtained from a model, from on- and off-line measurements and even historical process information in order to estimate important non-measurable variables. This is called a software sensor and has been successfully applied to bioreactor operation (Bastin and Dochain, 1990; Montague et al., 1992).

Once an estimation method is successfully developed in laboratory, there is still quite a long way to go when implementing it in a production environment. Such preconditions as user-friendliness, reliability and understandable functioning are hard to fulfil in industrial applications and may be even more difficult to realise than a well working algorithm (Halme, 1986). The above practical requirements are also relevant when selecting measurement techniques and instrumentation (Montague, 1997).

### 2.3 Modelling

Denn defines a model as a set of equations, the solution of which, given specified data, is representative of the process response to a corresponding set of inputs (Denn, 1986). Model equations can be obtained in several ways: using theoretical concepts, through direct experimental observation or by using the equations of a system believed to be analogous to the one under analysis. In general, considerable effort is required to establish useful models. Modelling can thus only be justified if the
models are less complicated than the processes they represent and lead to significant advantages (Lübbert and Simutis, 1994).

Process models have been widely used in the chemical industry, from the design phase to plant maintenance. Modelling biological processes is also commonplace. However, these models have limited use as they often lack robustness due to the processes not being fully understood. A brief survey of existing model types for biological processes will be presented below.

2.3.1 Modelling biological processes

Models of biological processes range from simple empirical descriptions of growth curves to models which include numerous, and sometimes complex, partial differential equations. The latter are capable of accounting for behaviour under a much wider variety of conditions (Fredrickson et al., 1967), but require detailed knowledge of the process.

Fredrickson and Tsuchiya were the first to categorise models according to the detail included in the system representation. These workers classified models into four distinct categories: unstructured, structured, unsegregated and segregated (Fredrickson and Tsuchiya, 1963).

Unstructured models describe the biophase only in terms of its quantity and not of its quality, whilst structured models require some qualitative description of the biophase (Harder and Roels, 1982). Unsegregated models consider the population of cells or microorganisms to be a single, uniform, lumped biophase (Fredrickson et al., 1970; Ramkrishna, 1979); these models are also called continuum or distributed models (Harder and Roels, 1982). Segregated models consider the existence of discrete, heterogeneous cells (Bailey and Ollis, 1986). Both unstructured and structured models may assume a segregated or unsegregated viewpoint. It is obvious that the 'real' situation is a structured, segregated viewpoint.
The simplest models are unstructured, distributed models (Harder and Roels, 1982) and are usually empirical descriptions of growth curves. A growth curve for a cell population in a conventional batch bioreactor usually exhibits four distinct periods (Bailey and Ollis, 1986):

- a lag period, during which very little or no growth occurs;
- an exponential period, during which rapid growth occurs, with the number of cells increasing exponentially with time;
- a stationary period, during which no growth occurs; and
- a decline period, during which the population dies.

The simplest model is known as Malthus’ Law and describes the exponential period only (Bailey and Ollis, 1986). This law assumes that the rate of increase of biomass is directly proportional to the current biomass. Although simple, it leads to the definition of the specific growth rate, of paramount importance in the description of biological processes. Modifications to Malthus’ Law have been proposed in order to incorporate other periods of the batch growth curve (Velhurst, 1838; Pearl, 1924, Volterra, 1959). Other workers suggested that the specific growth rate could be related to the concentration of a particular substrate in the growth medium (McKendrick and Pai, 1910; Monod, 1942; Monod, 1949). Monod adopted this approach to propose an empirical description of exponential growth in a fermentation process (Monod, 1942). This model is referred to as the Monod Equation and has been applied to the growth kinetics of many biological systems. Several modifications to the Monod Equation have been introduced to account for further features, e.g., the effect upon specific growth rate of cell concentration (Contois, 1959), substrate inhibition (Andrews, 1968; Aiba et al., 1968) and multiple growth limiting substrates (Megee et al., 1972; Ryder and Sinclair, 1972).

Unstructured, distributed models include two limiting assumptions: an ‘average cell’ approximation and an average biophase state approximation. As a result, it has been suggested that the use of these models should be restricted to a balanced growth situation (Bailey and Ollis, 1986). This is a growth state in which every extensive
property of the growing system changes by the same factor (Campbell, 1957). The most successful application of these models has been observed in chemostats (Monod, 1950; Herbert et al., 1956; Powell, 1958); under steady state conditions, balanced growth seems a realistic proposition (Harder and Roels, 1982). The major advantage of unstructured, distributed models is that they generally give rise to relatively simple, linear differential equations, which can either be solved analytically or by simple numerical techniques (Faraday, 1994).

Structured models can generally be divided into two types (Harder and Roels, 1982): those which simply consider the primary metabolism and those which attempt to model the internal biochemistry in some detail. The former type can still be sub-divided into compartmental and cybernetic models. Compartmental models define a number of internal pools or compartments (Williams, 1967; Harder and Roels, 1982). Cybernetic models assume that through ‘natural selection’ microbes have developed the ability to control their regulatory processes and, as a result, they are able to optimise their growth patterns (Ramkrishna, 1982; Ramkrishna et al., 1987). Structured models which describe the internal biochemistry are less common than compartmental or cybernetic models due to the large number of equations and parameters which must be considered (Harder and Roels, 1982).

Segregated models consider cells as individual and heterogeneous entities (Bailey and Ollis, 1986). As a result, segregation enables uncoupling reproduction from growth, i.e., whereas unsegregated models consider the biophase as a lump which ‘grows’, segregated models consider that the biophase changes due not only to cells growing, but to cells reproducing. These models are, thus, more complicated than unsegregated models and result in more complex differential equations to solve, normally requiring quite complex numerical methods (Fredrickson et al., 1967; Ramkrishna, 1979; Faraday, 1994).

Reproduction may be described either deterministically or stochastically. The deterministic approach considers cell division to be an explicit function of the state of
the system, only independent of time, whereas the stochastic approach considers cell division to be a partly random process (Faraday, 1994).

Deterministic descriptions for reproduction have proved inadequate for dealing with small populations of cells (Ramkrishna, 1979), whereas probabilistic functions have been successfully employed to describe the behaviour of cells with regard to their age distribution. This was possible with the introduction of the concept of population balance modelling (Fredrickson and Tsuchiya, 1963). Fredrickson and Tsuchiya derived the population age distribution considering a discrete semi-empirical description of a cell in terms of its age; they defined cell age as the length of time elapsed since the birth of that cell (Fredrickson and Tsuchiya, 1963). This approach has later been applied by other researchers (Shah and Borwanker, 1976; Faraday, 1994). As a result of these works, generic structured, segregated modelling frameworks have also been proposed (Fredrickson et al., 1967; Faraday, 1994).

### 2.3.2 Alternative approaches for modelling biological processes

As stated in Section 2.2.2, models may be used to estimate process information on-line and continuously (Stephanopoulos and San, 1984; Bastin and Dochain, 1990; Montague et al., 1992). However, mechanistic models such as those described above are often too complicated to be employed (Montague, 1997). Data-based and simple qualitative models, such as artificial neural network (ANN) models and fuzzy models, have been receiving considerable attention in the last decade. Although introduction of such models has increased the possibilities to describe biological processes, the models developed are simple descriptions and cannot be classified according to Fredrickson and Tsuchiya (Fredrickson and Tsuchiya, 1963).

ANNs are computational instruments that try to emulate the topology of the human brain, on an extremely simplified scale (Lippmann, 1987; Gomm et al., 1993; Zorzetto, 1995; Montague, 1997). They are also referred to as connectionist models because they are composed of a set of interconnected process units, called neurons or
nodes (Zorzetto, 1995). ANNs have the potential to identify and learn correlative patterns between sets of input data and corresponding target values (Huber et al., 1991); the latter are usually key process variables which are difficult to measure (Montague and Morris, 1994). Although a reasonable amount of representative data is required, ANNs offer a ‘black-box’ approach, being able to model relationships in a process with no knowledge of its mechanism. ANNs are thus very attractive when dealing with biological processes (Lant et al., 1988; Bulsari and Saxén, 1994) as these are often not well understood and normally result in a large amount of reliable process data, albeit mostly off-line.

Further modelling alternatives include fuzzy logic and neurofuzzy models. Fuzzy logic attempts to represent reality by introducing the idea of gradations of truth, or falseness (Postlethwaite, 1990); it thus ‘humanises’ conventional logic (Lübbert and Simutis, 1994). Fuzzy models are useful for non-linear processes (Sanders, 1998), where human experience outbalances mathematical modelling (Rhinehart and Murugan, 1996). Neurofuzzy models offer the precision and learning capability of ANNs, whilst being easy to understand, like fuzzy models (Lübbert and Simutis, 1994). Although widely used in fields such as robotics and financial markets, application of these models to biological systems is yet scarce (Kennedy and Spooner, 1996; Frank and Köppen-Seliger, 1997).

Combination of ANNs and fuzzy principles with poor process models provides an intermediate solution to modelling. These combined models are often referred to as hybrid or ‘grey-box’ models and have been successfully applied to biological systems (Gehlen et al., 1992; Wu and Joseph, 1992; Zorzetto, 1995; Fu and Barford, 1996; Shimizu, 1996; Groep, 1997).

2.4 Process Control

Chemical processes have been successfully controlled for many years and control
concerns are now present from the design phase. However, bioreactor control has encountered some limitations, mainly due to the nature of biological systems. A brief survey of the work reported on the application of conventional and alternative control strategies to chemical reactors and, especially, to bioreactors will be presented in this section, as well as some considerations on their implementation to a real environment. First, however, some fundamental control concepts will be briefly addressed.

2.4.1 Conventional control

There are several conventional control configurations; the most common is the feedback control configuration. In a simple feedback loop (Stephanopoulos, 1985; Seborg et al., 1989), the output of a process subject to disturbances is measured and compared to its desired value, i.e., the set point. A controller uses the difference between those values (the set point error) to act on a final control element, usually a valve. This will result in a change in the manipulated variable, driving the process output to its set point; this is referred to as closed-loop control (Stephanopoulos, 1985; Seborg et al., 1989).

A feedback controller can relate set point error and manipulated variable in different ways. There are three basic types of control action: proportional, integral and derivative. The action of a controller with all those actions, referred to as a PID controller, is given by (Stephanopoulos, 1985; Seborg et al., 1989):

\[
m(t) = m_b + K \left[ e_{SP}(t) + \frac{1}{\tau_I} \int_0^t e_{SP}(t) \, dt + \tau_D \frac{de_{SP}(t)}{dt} \right]
\]

(2.1)

where \( m(t) \) is the manipulated variable at time \( t \); \( m_b \) is the bias value, i.e., the actuating signal when \( e_{SP}(t) \) is zero; \( e_{SP}(t) \) is the set point error at time \( t \); \( K \) is the proportional gain; \( \tau_I \) is the integral action time and \( \tau_D \) is the derivative action time. \( K, \tau_I \) and \( \tau_D \) may be referred to as the controller parameters.
There are two distinct types of control problems, often referred to as servo and regulator problems (Stephanopoulos, 1985). In the former, the disturbances to the process remain constant, whilst set point changes; thus, the feedback controller acts so as to keep the process output close to the changing set point. In the latter, the set point remains the same, while the disturbances change; in this case, the feedback controller acts in order to eliminate the influence of those changes, keeping the process output at the set point.

In principle, the application of feedback control is straightforward, both for servo and regulator problems. However, there are some limitations in its use, e.g., no corrective action occurs until the controlled process output deviates from the set point or when the process output cannot be measured. In such cases, more advanced control strategies are necessary to effectively control the process. These include cascade, feedforward and ratio control.

Cascade control consists of using multiple feedback loops and is particularly useful when the disturbances are associated with the manipulated variable or when the final control element exhibits non-linear behaviour (Seborg et al., 1989). In feedforward control, the controller uses measurements of important disturbances to act on the process before the disturbances upset it (Stephanopoulos, 1985; Seborg et al., 1989). In practical conditions, feedforward control is normally used in combination with feedback control; this is referred to as feedforward-feedback control. Ratio control is a specific type of feedforward control, where the objective is to maintain the ratio of two variables, usually flow rates, at a specified value (Stephanopoulos, 1985; Seborg et al., 1989). Although these advanced control strategies are minor modifications of basic closed-loop control, their use can result in significant benefits (Montague, 1997).

In a situation with unknown or time varying variables, it may be advisable to consider adaptive control, which allows the modification of the controller coefficients to improve control action (Jacobs, 1981; Signal and Lee, 1992; Tong and Bobis, 1993). There are two different mechanisms for adaptation of the controller parameters:
programmed adaptive control and self-adaptive control (Stephanopoulos, 1985; Seborg et al., 1989; Bastin and Dochain, 1990). The former is comparable to feedforward-feedback control and is used when the process is well known and a process model is available. Otherwise, self-adaptive control may be used. It consists of two loops: a conventional feedback control loop and another loop which adjusts the controller parameters using an estimator. Several self-adaptive control systems have been successfully applied over the years to both chemical and bio-reactors (Dochain and Bastin, 1984; Jones et al., 1992; Zeng et al., 1992; Zeng et al., 1993a; Zeng et al., 1993b).

2.4.2 Control of bioreactors

Bioreactor control is a complex problem due to inherent non-linearities and time varying characteristics of the processes involved (Montgomery, 1986). The application of conventional controllers provides poor control due to problems in tuning controller parameters. Advanced, conventional control strategies result in better bioreactor control, although limitations are still observed due to the lack of reliable instrumentation for on-line measurement. Additional considerations related to bioreactor control are the selection of sampling frequency and the presence of noise. The dynamics involved in bioreactors are such that, if sampling frequency is too low, important phenomena may be missed out, even if on-line measurement is possible. This may result in the monitored data being of little or no use for control purposes (Montague, 1997). Furthermore, noise is usually present in these measurements, which prevents the use of derivative control action (Section 2.4.1).

In industrial applications, the control of bioreactors is usually restricted to the regulation of pH, temperature, pressure or CO₂ and O₂ in the exhaust gas (Heinzle et al., 1990; Bastin and Dochain, 1990; Ramseier et al., 1993) as these quantities can be measured on-line, easily and accurately (Section 2.2.1). Off-line measurements do not provide adequate process monitoring in bioreactors due to the fast dynamics of the
processes involved, which results in the information gathered often being outdated and, thus, useless for control purposes.

It is clear that bioreactor control could be more efficient if important process variables could be monitored. As stated in Section 2.2.2, models may be used to provide process information which cannot be measured easily, or at all. Incorporation of this information into a control algorithm could result in enhanced bioreactor control; this alternative control strategy is referred to as model-based control (Seborg et al., 1989; Montague, 1997).

2.4.3 Application of model-based control

Control algorithms incorporating process models have been developed for the chemical and process engineering industry since the early 1980s, when the concept of model-based control was introduced. Almost invariably, linear models were used; non-linear models were only later directly incorporated in control algorithms (Choi and Rhinehart, 1997). A generic model control (GMC) framework has been proposed by Lee and Sullivan, in which either linear or non-linear models can be employed (Lee and Sullivan, 1988). A number of examples have been reported to demonstrate the efficiency of this generic approach (Lee and Sullivan, 1988; Signal and Lee, 1995).

Several advanced model-based control strategies have been proposed by altering the 'basic' structure. These include using more complicated models, usually non-linear, and models based on ANNs and fuzzy logic; advanced, conventional control concepts, such as feedforward and adaptive control have also been applied. Recently, another concept has been introduced: model-based observer (MBO) control (Jones and Gawthrop, 1992; Gawthrop and Ponton, 1996). In MBO control, the model used to estimate important process information on-line can be updated by manipulating model parameters (Jones and Gawthrop, 1992; Tong and Bobis, 1993; Gawthrop and Ponton, 1996); this is particularly useful for processes in which behaviour is time dependent. MBO control has been applied to simple process engineering problems, such as flash
separation (Jones and Gawthrop, 1992) and the control of tanks in series (Gawthrop and Ponton, 1996).

Model-based control systems are now widely used for chemical processes; surveys of the major action areas can be found elsewhere (Bosley et al., 1991; Flaus et al., 1991). However, application of model-based control may be hindered by the presence of modelling errors, i.e., errors in numerical assumptions on the state of the process. Modelling errors include errors in: disturbances, initial model states, model parameters and model structure (Gawthrop and Ponton, 1996). The most important and frequent are the latter two, also referred to as parameter mismatch and structural mismatch (Signal and Lee, 1992). In general, all models suffer from these two types of mismatch, which affect controller performance; the ability of a controller to perform despite mismatch is called robustness (Signal and Lee, 1992).

It has been observed that the effect of structural mismatch can be made negligible in MBO control by updating specific model parameters (Signal and Lee, 1992). The use of feedback to drive a model towards a process despite modelling error is one approach to achieve robustness of the controller (Gawthrop and Ponton, 1996). Using system identification to reduce modelling error is another possibility (Stephanopoulos and San, 1984; Bastin et al., 1992).

Model-based control has also been adopted for bioreactor operation, following the success observed for chemical processes. It has been commented (Fredrickson et al., 1970) that all models of biological processes have potential use in control. However, some are not robust enough to meet control requirements due to lack of detailed process knowledge. Despite this limitation and the presence of modelling errors, model-based control has encountered wide applicability (Williams et al., 1986; Bosley et al., 1991; Van Impe, 1996); this has been attributed to the continuous estimation of important process information, which would otherwise be unavailable. Both linear (Williams et al., 1986) and non-linear models (Ramseier et al., 1993; Zeng et al., 1993a; King, 1997) have been employed. Furthermore, adaptive control strategies have also been developed using non-linear models (Zeng et al., 1992) and through the
use of ANNs and fuzzy logic systems (Aynsley et al., 1993; Lübbert and Simutis, 1994; Turner et al., 1996). However, until this work, application of MBO control to bioreactors has not been reported; this is mainly due to the unavailability of predictive and robust models.

2.4.4 Implementation of advanced control strategies

Overall, development of control strategies has become a search for more robust, advanced strategies, but also for generic methodologies. Although these methodologies have a wide range of application, judging on the number of publications reported, they do not always easily allow inclusion of system-specific information into the control design. Therefore, control theory has deviated from its prime objective - the control of physical systems; the lack of system-specific information may jeopardise or restrict the implementation of advanced control strategies (Costello and Gawthrop, 1997). Physical-model based control (PMBC) is a novel approach to using such system-specific information (Gawthrop, 1995; Costello and Gawthrop, 1997), particularly useful for partially known or non-linear systems. Experimental demonstration of the application of PMBC has already been reported (Maher et al., 1995; Costello and Gawthrop, 1997).

Many advanced control strategies work well in concept or simulation, but their implementation into a real operating environment may prove unsuccessful (Sanders, 1998). The reasons for this failure have been analysed by Sanders, and include (Sanders, 1998): instrument failures, lack of maintenance or long-term support by the strategy developers, not anticipating future changes in the operating range and how this would affect the efficiency of the strategy. An important finding of this review was that the subsequent implementation of advanced control strategies should be considered when still developing the strategies in order to take into account not only the process, but also exterior factors such as aging of instrumentation or the increasing push for higher production rates, mainly due to market competition and customer demands.
Recognition and identification of the difficulties involved in the implementation of advanced control strategies will certainly drive control towards an even more multi-disciplinary field, also bringing academia and industry closer. This may, in turn, contribute to control not being viewed in industry as a ‘mystic’ art (Montague, 1997), but as a necessary, reliable tool.

2.5 Concluding Comments

A brief survey of the literature in three areas has been presented in this chapter, namely: monitoring, modelling and process control. This survey illustrates the state-of-the-art of research in those areas, focusing on applications to bioreactors, and sets the foundation for the work presented in the rest of this dissertation.

It is clear from this survey that bioreactor control exhibits limitations when employing conventional strategies, which are mainly due to the lack of reliable instrumentation. Advances in monitoring have been reported, although there is an increasing use of dynamic models to estimate important process information on-line and thus help controlling processes. The latter approach is receiving a lot of attention for bioreactor operation, and several workers have reported successful applications of model-based control. The models used range from simple process descriptions to more complicated models; the application of ANNs and fuzzy logic in modelling has also been demonstrated to be useful in some circumstances. Finally, it is also clear that implementation of advanced control is not always a straightforward procedure due to factors external to the process and the bioreactor, and that such considerations should be considered when still developing the control strategy.

Recent applications of models in control include the development of model-based observer (MBO) control. However, until this work, no work has been reported on the application of this control strategy to bioreactor operation. The model used in this work was a structured, segregated model, which is the most accurate type of model for
biological processes. This model was used as an observer to estimate important information on-line. Successful application of this control strategy will not only result in enhanced process performance, but in enhanced understanding of the process dynamics due to the detail included in the process model. The rest of this dissertation will address the development and application of MBO control to a simulated bioreactor. First, though, some background information on the propagation methods considered, the model used as an observer and a more detailed description of MBO control will be presented.
Chapter 3
Background

3.1 Introduction

Application of conventional control to bioreactors is often poor, mainly due to the lack of reliable instrumentation for on-line monitoring. An alternative approach is to use a model to estimate on-line information which would otherwise be difficult or impossible to obtain. An overview of the literature on monitoring, modelling and their use in bioreactor control has been presented in Chapter 2. It is clear from this survey that there is a need for more efficient control strategies for bioreactor operation. In this dissertation, the application of model-based observer control to bioreactors is considered.

In this chapter, the principle of model-based observer control will be outlined. The model employed as an observer in this strategy will be described and the propagation methods considered for the application of the control strategy will be introduced. Finally, some model predictions obtained for the propagation methods selected will also be presented and analysed.

3.2 Model-based Observer Control

The principle of model-based observer (MBO) control is illustrated in Figure 3.1. Two distinct feedback loops can be identified: the process loop in blue and the model loop in green. These loops are interconnected as indicated in red; disturbances to the system are shown in brown.

In the process loop, the process controller manipulates process parameters according
to the difference between a set point and a process output (the set point error). The process is thus altered so that its output is driven towards the set point. In the model loop, the model adaptor modifies model parameters dependent upon the difference between the 'desired' model output and its actual value. The purpose of the model adaptor is to modify parameters in the model so that its output is driven towards the 'desired' value.

The interconnection between the process and model loops allows information from the model to be used by the process controller and the model to be updated on the basis of actual process behaviour. The disturbances to both process and model may be considered to be the same and, thus, process and model outputs should be the same if the model is a good representation of the process. The model infers some process information which is otherwise unavailable on-line or difficult to obtain (Gawthrop and Ponton, 1996); in this case, the model is said to act as an observer. These non-measurable model outputs are used as additional information to the process controller with the purpose of enabling it to enhance process performance more efficiently. As can be seen in Figure 3.1, the process parameter thus manipulated is employed as an input not only to the process, but also to the model. The model is updated by comparing the model output to the process output, which is the 'desired' value; thus, process/model mismatches can be identified and corrected by the model adaptor.

The above description of MBO control differs slightly from the approach adopted by other workers (Jones and Gawthrop, 1992; Gawthrop and Ponton, 1996). These workers do not consider the process outputs for the process controller action; instead, both measurable and non-measurable model outputs are considered and compared to a set point. Furthermore, they refer to the model adaptor as estimator (Jones and Gawthrop, 1992) or model controller (Gawthrop and Ponton, 1996). The terminology used in this dissertation emphasises the corrective nature of the model loop by referring to the estimator or model controller as a model adaptor. In conventional control, adaptation refers to the adjustment, or tuning, of controller coefficients with the purpose of enhancing the control action and in order to compensate for variations
in the process dynamics (Stephanopoulos, 1985; Seborg et al., 1989). In this dissertation, adaptation refers to the manipulation of model parameters with the purpose of driving the model towards the process, i.e., to ‘adapt’ the model on-line. The model adaptation procedure is independent of potential adaptive tuning of the control parameters of the process controller and/or the model adaptor.

It is clear that implementation of MBO control requires: a control algorithm for the process controller; a control algorithm for the model adaptor; and a robust and predictive process model. The development and testing of the algorithms and their integration into the overall MBO control algorithm will be addressed in Chapters 4 to 7. In this chapter, the process model used will be introduced in Section 3.4 and some model predictions will be presented in Section 3.5. First, however, the various propagation methods employed in bioreactor operation which may require process control will be presented in Section 3.3.

3.3 Propagation Methods

Bioreactors can be operated in either batch or continuous mode; different propagation methods can be employed in either mode. Conventional batch and fed-batch operation are batch propagation methods; repeated batch, repeated fed-batch, semi-continuous, chemostat, perfusion and perturbed-feeding operation are examples of continuous propagation methods.

In conventional batch operation, nothing is added to, or removed from, the culture once the run is started, except for monitoring purposes; death is inevitable due to the build up of toxic components. Fed-batch propagation methods allow this build up to be controlled through periodic feeding of nutrients. However, no components are removed from the bioreactor; thus, death is delayed, but inevitable.

Continuous propagation may involve the discontinuous or continuous addition and/or
removal of components from the bioreactor. Repeated batch, repeated fed-batch and semi-continuous operation are examples of discontinuous propagation methods; chemostat, perfusion and perturbed-feeding operation are examples of continuous propagation methods.

The principle of repeated batch operation is to harvest a known fraction of the bioreactor contents (the harvest fraction, HF) at regular intervals (the dilution cycle time, DCT). The remaining bioreactor contents are diluted with fresh growth medium and operated in batch mode until the next resuspension. DCT should be such that resuspension occurs in the late exponential period and HF should be such that the cell concentration at the beginning of each cycle is similar to that of the first resuspension cycle. In repeated fed-batch, components are added to the culture in between resuspensions, although removal of components only happens at the end of each cycle. In semi-continuous operation, a much smaller fraction of the bioreactor contents is harvested and new medium is added on a more frequent basis than in repeated batch.

In chemostat operation, there is a continuous feed of medium to the bioreactor, which is balanced by the removal of bioreactor contents; the bioreactor is designed to operate at steady state. The name ‘chemostat’ is due to the growth rate of the culture being controlled by its chemical environment, i.e., the availability of a limiting component in the medium (Stanbury and Whitaker, 1987). Perfusion propagation methods involve the continual feeding of growth medium and removal of sterile bioreactor contents; cells are not removed from the bioreactor at any stage. Perturbed-feeding operation involves periodically varying one of the feed stream parameters, typically the dilution rate or a nutrient concentration.

In this dissertation, conventional batch and repeated batch operation will be considered for the application of the MBO control strategy. Batch mode is the most common bioreactor operation used in industry; conventional batch operation has been considered as it is the simplest batch operation. Repeated batch operation has been selected as it is also widely used in industry, mainly due to being interpreted as an ‘extension’ of batch operation, i.e., a ‘continuous’ series of batch runs.
3.4 The Model

The modelling framework employed has previously (Faraday, 1994) proved to be a robust and predictive modelling tool; it is based on the cell cycle as proposed by Howard and Pelc (Howard and Pelc, 1953). Specific models for specific cell lines can be built within this framework. In order to develop the control algorithm necessary for the application of the MBO control strategy, a specific model has been used which encompasses most of the framework features. The modelling framework and the specific model will be briefly presented in Sections 3.4.1 and 3.4.2, respectively.

3.4.1 The modelling framework

CELCYMUS (Cell Cycle Model, University of Surrey) is a generic modelling framework developed by Faraday and Kirkby (Faraday, 1994). It is a structured and segregated model which describes the cell population in terms of a cell age distribution. In CELCYMUS, the inter-mitotic period is divided into distinct growth phases and any number of phases may be defined; these are referred to as cell cycle phases, although they may differ from those described by Howard and Pelc (Howard and Pelc, 1953). Cells in different phases may interact with the growth medium independently of the behaviour of cells in other phases. Furthermore, cells in different phases and of different ages within a phase may have different cytological states, i.e., the concentration of the intra-cellular components may differ.

The biological age, $\tau$, describes the cell age within a phase. The cell age distribution is the position of all cells, in all phases, at any instant. Changes in the cell age distribution are the result of cells being washed out of the bioreactor, progressing through a phase (flow) or moving to another phase (transition). Transition can occur at any point within a phase and is dependent on the transition rules defined for that phase, which may be stochastic, deterministic or a combination of both. These transition rules may be dependent upon: biological age, the concentration of any of the components of the medium or the concentration of any of the intra-cellular
components. All phases have at least one transition rule, which dictates that all cells must leave the phase when the maximum age allowable for that phase is reached.

The cell age distribution for an arbitrary phase $X$, at time $t$, is denoted by $n_X(t, \tau_X)$; this is a population density function defined as the number of cells per unit volume of bioreactor, per unit biological age in phase $X$. The rate of change of the cell age distribution in phase $X$ with respect to time, assuming a well-mixed reactor, is mathematically given by:

$$
\frac{\partial n_X(t, \tau_X)}{\partial t} = \frac{F_I(t)}{V(t)} \left[ n_{X_I}(t, \tau_X) - n_X(t, \tau_X) \right] - n_X(t, \tau_X) \sum_{j=1}^{G_X} F_{JX} \left[ \tau_X, C_{CX}(t, \tau_X), C(t) \right] - \frac{\partial n_X(t, \tau_X)}{\partial \tau}
$$

(3.1)

where $F_I(t)$ is the inlet flow rate at time $t$ (m$^3$ h$^{-1}$); $V(t)$ is the working volume of the bioreactor at time $t$ (m$^3$); $\tau_X$ is the biological age in phase $X$ (h); $n_{X_I}(t, \tau_X)$ is the inlet feed concentration of cells of age $\tau_X$ to $\tau_X + d\tau$, at time $t$ (cell M$^{-3}$ h$^{-1}$); $G_X$ is the number of different transition rules defined for phase $X$, of which $F_{JX}$ is the $j^{th}$ rule; $C_{CX}(t, \tau_X)$ is the cytological state vector at time $t$ (kg m$^{-3}$ cell$^{-1}$) and $C(t)$ is the medium state vector at time $t$ (kg m$^{-3}$). The cytological state vector and the medium state vector record the concentrations of the intra-cellular components and the medium components, respectively.

The mass balance for any component $K$ in the medium includes a term for the washout of the bioreactor and another for the net transport rate of component $K$ into a cell, for all phases. Mathematically, the rate of change of the concentration of component $K$ in the medium is given by:
Background

\[ \frac{dC_K(t)}{dt} = \frac{F_1(t)}{V(t)} \left[ C_{IK}(t) - C_K(t) \right] \]

\[ \quad - \sum_{X=1}^{N_p} \left\{ P_{KX} \left[ C_K(t), C_{CKX}(t, \tau_X), R_{KX} \right] \int_0^{T_X} n_X(t, \tau_X) \, dt \right\} \]  (3.2)

where \( C_K(t) \) is the concentration of component \( K \) in the medium at time \( t \) (kg m\(^{-3}\)); \( C_{IK}(t) \) is the inlet concentration of component \( K \) in the medium at time \( t \) (kg m\(^{-3}\)); \( C_{CKX}(t, \tau_X) \) is the intra-cellular content of medium component \( K \) for all cells of age \( \tau_X \), in phase \( X \), at time \( t \) (kg m\(^{-3}\) cell\(^{-1}\)); \( P_{KX} \) is the average net transport rate per cell of component \( K \) for a cell in phase \( X \) (kg cell\(^{-1}\) h\(^{-1}\)); \( R_{KX} \) is the rate constant for the transport of component \( K \) into a cell in phase \( X \) (units dependent upon transport kinetics); \( N_p \) is the total number of cell cycle phases considered and \( T_X \) is the maximum biological age a cell can attain in phase \( X \) (h).

The mass balance for any intra-cellular component \( L \) includes a term for the net transport rate of component \( L \) into a cell and another for the net internal production rate per cell of intra-cellular component \( L \). Mathematically, the rate of change of the intra-cellular concentration of component \( L \) is given by:

\[ \frac{dC_{CLX}(t, \tau_X)}{dt} = P_{LX} \left[ C_L(t), C_{CLX}(t, \tau_X), R_{LX} \right] + P_{CLX} \left[ C_{CX}(t, \tau_X), R_{CX} \right] \]  (3.3)

where \( C_{CLX}(t, \tau_X) \) is the intra-cellular content of component \( L \) for all cells of age \( \tau_X \), in phase \( X \), at time \( t \) (kg m\(^{-3}\) cell\(^{-1}\)); \( P_{LX} \) is the average net transport rate per cell of component \( L \) for a cell in phase \( X \) (kg cell\(^{-1}\) h\(^{-1}\)); \( C_L(t) \) is the concentration of component \( L \) in the medium at time \( t \) (kg m\(^{-3}\)); \( R_{LX} \) is the rate constant for the transport of component \( L \) into a cell in phase \( X \) (units dependent upon transport kinetics); \( P_{CLX} \) is the average net internal production rate per cell of intra-cellular component \( L \) (kg cell\(^{-1}\) h\(^{-1}\)); \( C_{CX}(t, \tau_X) \) is the cytological state vector at time \( t \) (kg m\(^{-3}\) cell\(^{-1}\)) and \( R_{CX} \) is the cytological rate constant vector in phase \( X \) (units dependent upon the interactions of the intra-cellular components).
Solution of the model is confined to solving Equations 3.1 to 3.3, for all phases and all medium and intra-cellular components. Equation 3.1 is a first order quasi-linear hyperbolic partial differential equation. However, application of the Method of Characteristics transforms it into an ordinary differential equation:

\[
\frac{dn_X(t, \tau_X)}{dt} = \frac{F_1(t)}{V(t)} \left[ n_X(t, \tau_X) - n_X(t, -rx) \right] \\
- n_X(t, \tau_X) \sum_{j=1}^{G_X} F_{jX} \left[ \tau_X, C_{CX}(t, \tau_X), C(t) \right]
\] (3.4)

A simple numerical technique such as Euler Integration can thus be used to solve the three sets of ordinary differential equations defined by Equations 3.2 to 3.4. However, the number of equations to be solved is potentially large and the order in which they must be solved is vitally important.

A more detailed description of CELCYMUS can be found elsewhere (Faraday, 1994), as well as a thorough analysis of results obtained with this generic model. That work demonstrated the predictive and robust nature of this generic cell cycle model.

### 3.4.2 The specific model

The model used as an observer in the MBO control strategy was built within CELCYMUS for the mm321 hybridoma cell line (Faraday, 1994). A schematic of this specific model is presented in Figure 3.2. The dark arrows represent the interactions of the population with the medium; the light grey arrows refer to random transitions. The experimental data necessary to develop this specific model were obtained by Hayter (Hayter, 1989).

The conceptual features of the specific model are the following:

- there are six phases - G1', G1, S, G2, M and D;
- the S phase is the DNA synthesis phase;
• the M phase is where mitosis occurs;
• G1 and G2 are the gap phases between the M and S phases;
• the G1 phase is divided into two distinct periods, referred to as G1a and G1b phases;
• the G1’ phase accounts for the initial lag period and cells in this phase enter the cell cycle via G1b;
• cells in the D phase are still viable, although irreversibly out of the cell cycle and approaching death;
• in principle, cells progress around the cell cycle in the following order - G1a, G1b, S, G2, M and back to G1a;
• the G1a, S, G2, M and G1’ phases are of fixed duration - 2.5, 5, 2, 2 and 14 h, respectively;
• the G1b and D phases are of variable duration, with maximum durations of 10 and 50 h, respectively;
• glutamine is consumed at a fixed rate by cells in the G1a and G1b phases;
• the consumption of glutamine by chemical hydrolysis is also taken into account;
• ammonia is produced at a rate which is proportional to the rate of assimilation of glutamine and is excreted to the medium in the G1a and G1b phases;
• glucose is assimilated in the G1a, G1b, S and G2 phases at a rate which is proportional to the glucose concentration in the medium;
• lactate is produced and excreted in the G1a, G1b, S and G2 phases at a rate which is proportional to the rate of assimilation of glucose;
• antibody is produced at a fixed rate during the G1b and S phases;
• the initiation of the S phase is controlled by a stochastic transition;
• the probability of this transition is dependent on the cumulative amount of glutamine of G1b cells;
• the relationship between the probability of this transition and the cumulative glutamine content is a quadratic form;
Background

- once cells enter the D phase, they are trapped in this phase and die with first order kinetics;
- the cell death rate is a function of the environmental conditions, being proportional to ammonia concentration raised to the power of 1.5;
- before glutamine exhaustion, cells which remain in G1b for the whole of its maximum duration will immediately enter D, instead of going into S; and
- in a glutamine free medium, cells which complete mitosis are incapable of initiating the G1a phase and enter the D phase. Cells which enter G1b after glutamine exhaustion will be trapped and will enter the D phase after the maximum duration of the G1b phase.

The concepts presented above are described by a set of equations; Euler Integration was employed to solve the model. Some of those equations are presented below due to their importance in the subsequent development of the MBO control algorithm. These refer to the changes in the concentrations of the medium components, the fraction of cells entering the S phase due to the stochastic transition and the death rate of cells in the D phase.

The expressions for the rate of change of glutamine, ammonia and antibody concentrations in the medium, for batch mode, can be derived from Equation 3.2 and are as follows:

\[
\frac{dC_{\text{Glut}}(t)}{dt} = - R_{\text{Glut}} \left[ \int_{0}^{T_{G1a}} n_{G1a}(t, \tau_{G1a}) d\tau_{G1a} + \int_{0}^{T_{G1b}} n_{G1b}(t, \tau_{G1b}) d\tau_{G1b} \right] \quad (3.5)
\]

\[
\frac{dC_{\text{Ammo}}(t)}{dt} = 0.1 R_{\text{Glut}} \left[ \int_{0}^{T_{G1a}} n_{G1a}(t, \tau_{G1a}) d\tau_{G1a} + \int_{0}^{T_{G1b}} n_{G1b}(t, \tau_{G1b}) d\tau_{G1b} \right] \quad (3.6)
\]

\[
\frac{dC_{\text{Anti}}(t)}{dt} = R_{\text{Anti}} \left[ \int_{0}^{T_{G1b}} n_{G1b}(t, \tau_{G1b}) d\tau_{G1b} + \int_{0}^{T_{S}} n_{S}(t, \tau_{S}) d\tau_{S} \right] \quad (3.7)
\]
The glutamine consumption rate, $R_{Glut}$ (kg h$^{-1}$ cell$^{-1}$), is assumed to be zero order and constant throughout the G1a and G1b phases. The antibody production rate, $R_{Anti}$ (kg h$^{-1}$ cell$^{-1}$), is also assumed to be zero order and constant throughout the G1b and S phases. It is assumed that ammonia is produced in the same phases as glutamine is assimilated and that, overall, 0.1 kg of ammonia are produced per kg of glutamine. Glucose and lactate were not considered as part of this work.

The relationship between the fraction of cells to have initiated the S phase due to the stochastic transition and the cumulative glutamine content of those cells is given by:

$$\frac{n_{G1b}(t, \tau_{G1b})}{n_{G1b}(t - \tau_{G1b}, 0)} = \left[ \frac{C_{GlutG1b}(t, \tau_{G1b}) - S_{Max}}{S_{Max}} \right]^2$$  

where $n_{G1b}(t, \tau_{G1b})$ is the concentration of cells of age $\tau_{G1b}$ at time $t$ (cell m$^{-3}$ h$^{-1}$); $n_{G1b}(t - \tau_{G1b}, 0)$ is the concentration of cells which entered phase G1b at time $t - \tau_{G1b}$ (cell m$^{-3}$ h$^{-1}$); and $S_{Max}$ is the maximum cumulative glutamine content a cell may obtain in G1b before it has to initiate the S phase (kg cell$^{-1}$).

It is assumed that once cells enter the D phase they die with first order kinetics, although the rate constant is dependent on ammonia. The rate of cell death, $r_D$ (cell m$^{-3}$ h$^{-1}$), is given by the expression:

$$r_D = k [C_{Ammo}(t)]^{1.5} \int_0^{T_D} n_D(t, \tau_D) d\tau$$  

where $k$ is the death rate constant (m$^3$)$^{1.5}$ kg$^{-1.5}$ h$^{-1}$; $C_{Ammo}(t)$ is the ammonia concentration in the medium at time $t$ (kg m$^{-3}$) and $T_D$ is the maximum duration of the D phase (h).

The specific model described in this section has proven successful in simulating and predicting the consumption and production kinetics and the growth of the mm321
hybridoma cell line over a range of process conditions (Faraday, 1994). This specific model has been chosen to act as an observer in the MBO control strategy. A copy of the model has been employed in the process loop, instead of a real process; this may be referred to as a 'pseudo-process'. This was possible due to the robustness of the model used. Furthermore, it would be too time consuming and unrealistically expensive to use a real bioreactor for the preliminary development and testing of a MBO control algorithm as there were no previous data or literature on the application of MBO control to bioreactors.

In this work, the measurable model and process outputs considered will be the viable cell, glutamine, antibody and ammonia concentrations. The copy of the model residing in the model loop will be used to predict cell age distribution on-line.

Cell age distribution is the position of all cells, in all phases, at any instant, as explained in Section 3.4.1. Cell cycle phases were divided into a large number of age elements (of length \( dt \)) in order to solve the model used. However, \( dt \) was considered to be a small number, which resulted in many elements in each phase. As such, the cell population was considered to be a large number of sub-populations. The position of all these sub-populations, at any instant, is clearly a great amount of information. In order to analyse and subsequently control cell age distribution, an alternative description would be desirable.

Experimentally, flow cytometry is used to measure cell age distribution in terms of cell cycle position (Bailey and Ollis, 1986). However, it can only resolve between cells with a single DNA copy, a double DNA copy or those in between; in other words, it distinguishes between G1+G0 cells, G2+M cells and S cells, where G0 is a quiescent phase. Therefore, in terms of the model used, it would not distinguish between cells in G1', G1a, G1b and D, either. For the purpose of this work, cell age distribution was defined as the percentage of cells in each phase of the model.
3.5 Model Simulations in Batch and Repeated Batch

Some simulations conducted with the specific model considered will be presented below so that process behaviour with no control action is known and understood. These model predictions were obtained considering batch or repeated batch operation.

3.5.1 Batch operation

A typical batch run was simulated for an initial viable cell concentration of $0.09 \times 10^{6}$ cell ml$^{-1}$ and an initial glutamine concentration of 0.1 mg ml$^{-1}$; all cells were initially in $G_1'$, evenly distributed throughout this phase. The viable cell and medium component concentrations and the viability obtained over 100 h are presented in Figure 3.3. It can be seen that the onset of cell death coincides with glutamine exhaustion. Ammonia and antibody concentrations exhibit the expected behaviour (Section 3.4.2), reaching a plateau when viability is low.

The cell age distribution obtained is presented in Figure 3.4. It can be seen that, at the beginning of the culture, $G_1'$ cells progressively enter $G_1b$. After $\approx 20$ h, the cell age distribution approaches an approximate steady state. Once glutamine is exhausted, there is an immediate increase of cells in D as cells reaching the end of M cannot progress further in the cell cycle. There is also an increase in the percentage of cells in $G_1b$ as cells cannot proceed to the S phase, accumulating in $G_1b$ for its maximum duration before entering the D phase. As a result, a decrease is observed in cells in $G_1a$ and S, subsequently corresponding to decreases in G2 and M. It can be seen in Figure 3.4 that, by 70 h, all viable cells are in the D phase, at which point the viability is only $\approx 16\%$ (Figure 3.3).

Simulations have also been conducted at higher initial glutamine concentrations, for the same inoculum conditions. The viable cell and medium component concentrations and the viability obtained over 150 h, for an initial glutamine concentration of 5 mg ml$^{-1}$, are presented in Figure 3.5. It can be observed that only 92% of the initial
glutamine has been consumed after 150 h and that viability remains at a high value. The cell age distribution is presented in Figure 3.6; it is very similar to that in Figure 3.4 for the initial 50 h, as expected. After about 50 h, variations in the percentage of cells in each phase are minimal, except for the D phase; this is due to glutamine being present in excess.

### 3.5.2 Repeated batch operation

Repeated batch runs were simulated at an initial viable cell concentration of $0.09 \times 10^6$ cell ml$^{-1}$ and an initial glutamine concentration of 0.1 mg ml$^{-1}$, for the same inoculum conditions considered for batch operation. The viable cell concentration and the viability obtained at a harvest fraction (HF) of 80% and a dilution cycle time (DCT) of 50 h, over 100 resuspension cycles, are presented in Figure 3.7; only the initial and final viable cell concentrations and the final viability are shown for each cycle. It can be seen that there is little cycle to cycle variation in the final cell concentration; the viability remained constant and at a high value throughout the run. Glutamine, ammonia and antibody concentrations are not presented, but their variation with time exhibited the expected behaviour.

An extract of the cell age distribution obtained is presented in Figure 3.8. The distribution obtained in the first cycle is very similar to that obtained for the initial 50 h in Figure 3.4, as expected. It can be seen that, after 300 h (6 cycles), the cell age distribution reaches a steady state; the distribution repeats itself, with a two-cycle period, which is also depicted in Figure 3.7.

### 3.5.3 Varying operational conditions in repeated batch

Problems have been identified and reported from industry with repeated batch operation, such as large variations in culture growth in consecutive resuspension cycles; these problems are usually attributed to the ‘variability’ of biological systems. Such scenarios have been successfully simulated and analysed previously (Faraday,
1994) using models built within CELCYMUS. This analysis has attributed these problems primarily to variations in the cell age distribution caused by the selection of HF and/or DCT. A comprehensive study of the effect of HF and DCT on process behaviour has also been undertaken in this work. Therefore, further simulations in repeated batch were conducted at the same previous initial conditions, but changing the operational conditions. Analysis of the results obtained led to the identification of four different types of behaviour: periodic, aperiodic, unrecoverable catastrophic failure and washout. Periodic behaviour was defined as the existence of a repetitive pattern in culture growth, over the total number of resuspension cycles; an example of this behaviour has been presented in Figure 3.7. The remaining behaviour types identified are illustrated below.

Aperiodic behaviour was defined as the lack of a discernable pattern in culture growth, over the total number of cycles considered. An example is presented in Figure 3.9, obtained at a HF/DCT pair of values of 70/50. A high degree of variation in both viable cell concentration and viability is observed between consecutive cycles. This is due to frequent glutamine exhaustion which led to catastrophic failure, i.e., to a decrease in cell growth within a cycle. An extract of the cell age distribution obtained is presented in Figure 3.10. It can be observed that there is a decrease in the percentage of G1b cells and an increase in the percentage of D cells immediately prior to the start of the third resuspension cycle. This is due to the high percentage of viable cells, which exhaust the glutamine in the medium. Therefore, it can be concluded that a HF value of 70% results in too many cells being left in the bioreactor which consume and, in some resuspension cycles, exhaust the glutamine present in the medium; a HF value of 80% proved to be high enough to prevent this situation (Figure 3.7). The decrease of cells in G1b as they enter D results in the subsequent variations observed for the percentage of cells in the other cell cycle phases. It takes about a further cycle for the percentage of cells to return to the previous levels, at which point the viable cell concentration increases within a cycle (Figure 3.9). A similar explanation applies to other points in the simulation where a decrease in the viable cell concentration is exhibited within a cycle.
Unrecoverable catastrophic failure is an extreme case of aperiodic behaviour, in which the system is unable to recover from the frequent decrease in cell growth within a cycle. The viable cell concentration and viability obtained at HF/DCT values of 80/65 are presented in Figure 3.11 to illustrate this behaviour. It can be seen that the exhibited behaviour is similar to that obtained for the 70/50 pair of values (Figure 3.9), for the initial 38 resuspension cycles. However, overall, the viable cell concentration is lower and, after 38 cycles, cells die. It can be seen in Figure 3.3 that 65 h allows G1b cells to exhaust glutamine, resulting in cells reaching the stationary period and the death period; cell death was delayed due to the high HF value used. An extract of the cell age distribution obtained is presented in Figure 3.12. It can be seen that after 38 cycles, all viable cells are in the D phase. The step-wise decrease in the percentage of D cells is due to the maximum duration of the D phase being set to 50 h; after this time, all cells are considered to be dead.

Washout was defined as the continuous decrease in culture growth between consecutive resuspension cycles, whilst maintaining high viability. The viable cell concentration and viability obtained for the 85/50 pair of values are presented in Figure 3.13, as an example of this behaviour. It can be seen that, by just over 20 cycles, there are very few cells present, although these are extremely viable, more than for the 80/50 pair of values (Figure 3.7). Most cells are, thus, being washed out of the bioreactor; the remaining cells are extremely viable as glutamine exhaustion does not occur. An extract of the cell age distribution obtained is presented in Figure 3.14. It is very similar to that in Figure 3.8, but smaller variations in the percentage of cells in each of the phases are observed. Furthermore, the percentage of cells in the D phase is slightly higher throughout the simulation, approaching a steady state value after 600 h (12 cycles). Cells in the first cycle are enough to exhaust the glutamine present in the medium, resulting in cells entering the D phase. The percentage of D cells does not decrease within a cycle as much as for the 80/50 case because there are less cells present in the bioreactor. Therefore, the consumption of glutamine is smaller and the ammonia concentration in the medium is considerably smaller. The rate of cell death is thus small (Equation 3.9) and cells remain in the D phase for longer. This also explains the initial decrease in viability observed in
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Figure 3.13; after the initial 12 resuspension cycles, viability remains constant at a value of 93%.

It can be concluded from the above results that the model employed is capable of reproducing the results reported from industry (Faraday, 1994). Furthermore, the variations in culture growth between consecutive resuspension cycles are successfully interpreted by analysis of the cell age distribution obtained from the model.

3.6 Concluding Comments

The application of MBO control to bioreactors is proposed in this dissertation. The principle of this control strategy has been outlined in this chapter. The MBO controller modifies process outputs with respect not only to set point error, but also to additional information inferred on-line by the model, which would otherwise be unavailable. The controller thus requires a process loop and a model loop, which have to be interlinked. A conventional process controller resides in the process loop; a model adaptor resides in the model loop in order to correct any process/model mismatches on-line.

A robust and predictive process model is required in the model loop. A specific model built within a modelling framework (CELCYMUS) will be used in this work. A copy of the specific model will be employed in the process loop as a pseudo-process. Both the framework considered and the specific model used have been briefly described in this chapter.

Bioreactors can operate in either batch or continuous mode. In this work, conventional batch will be used as an example of batch mode and repeated batch as an example of continuous mode. These propagation methods were chosen for application of MBO control as they are the most commonly encountered in industry. Some model
Background

Simulations conducted in both batch and repeated batch were presented and briefly analysed in this chapter.

The application of MBO control requires algorithms for both process controller and model adaptor. The development and testing of these algorithms will be addressed in Chapters 4 to 6. These algorithms have to be integrated into an overall control algorithm in order to consider the interconnection between process and model loops. Testing of the overall MBO control algorithm will be presented and discussed in Chapter 7.
Chapter 4
Development and Testing of the 
Model Adaptor Algorithm

4.1 Introduction

In this dissertation, model-based observer (MBO) control is proposed to enhance process performance in a simulated bioreactor, operating in either conventional batch or repeated batch. Implementation of this control strategy requires a robust and predictive process model. The model used in this work has been introduced in Chapter 3; some model simulations conducted in batch and repeated batch were also presented and analysed. It is also necessary to develop an overall control algorithm, encompassing algorithms for both the model adaptor and the process controller. In this work, these algorithms were separately developed and tested prior to their integration into an overall MBO control algorithm.

In this chapter, the methodology employed for the development of the model adaptor algorithm will be detailed. The results obtained when testing the algorithm in batch operation will be presented and discussed; results obtained in repeated batch will be addressed in Chapter 5. Development and testing of the process controller algorithm and the MBO control algorithm will be addressed in Chapters 6 and 7, respectively.

4.2 Development of the Model Adaptor Algorithm

The purpose of the model adaptor algorithm is to drive the model towards the process, i.e., to adapt the model. This can be achieved by manipulation of the structure and/or the parameters of the model. The control algorithm developed considered only
Development and Testing of the Model Adaptor Algorithm

manipulation of model parameters, although some of these were directly related to the structure of the model. To test the algorithm, some model parameters were initially modified so that the model outputs would be different from the process outputs.

A block diagram of the model loop considered for the development and testing of the model adaptor control algorithm is presented in Figure 4.1; the grey lines refer to the elements of the overall MBO control strategy (Figure 3.1) which were not considered at this stage. The process controller was considered to be switched off; thus, the process was simulated at conditions for which no control was required. Furthermore, no unknown disturbances were considered as the process was simulated.

4.2.1 Control action

The model adaptor manipulated model parameters as follows:

\[ x_m(t) = x_b(t) + c_a(t) \]  \hspace{1cm} (4.1)

where \( x_m(t) \) is the current model parameter value (units as appropriate) and \( x_b(t) \) is the bias value (units of the model parameter). The definition of the term \( c_a(t) \) (units of the model parameter) depends on the control action considered for the model adaptor. Proportional-only and proportional-integral control actions were considered; a derivative term was not considered due to the usual presence of noise associated with bioreactor operation. For proportional-only action, \( c_a(t) \) is defined as:

\[ c_a(t) = K_a [ y_p(t) - y_m(t) ] \]  \hspace{1cm} (4.2)

and for proportional-integral action:

\[ c_a(t) = K_a \left[ y_p(t) - y_m(t) + \int_0^t (y_p(t) - y_m(t)) \, dt \right] \]  \hspace{1cm} (4.3)
Development and Testing of the Model Adaptor Algorithm

where $K_a$ is the proportional gain of the model adaptor (units defined by the units of the model parameter and of the outputs); $\tau_a$ is the integral action time of the model adaptor (units defined by the units of the model parameter and of the outputs); $y_p(t)$ is the current process output (units as appropriate) and $y_m(t)$ is the current model output (units as appropriate). $K_a$ and $\tau_a$ will be referred to as the model adaptor parameters.

Six model parameters were chosen to be manipulated so as to fully explore the model used. These were:

- the specific glutamine uptake rate, $R_{Glut}$;
- the specific antibody production rate, $R_{Anti}$;
- the maximum amount of glutamine a cell can consume during the G1b phase, before it has to proceed to the S phase, $S_{Max}$;
- the death rate constant, $k$;
- the duration of the G1a phase, $T_{G1a}$; and
- the duration of the S phase, $T_S$.

These parameters were introduced and defined in Section 3.4.2; the values used to simulate the process are presented in Table 4.1.

<table>
<thead>
<tr>
<th>$R_{Glut}$ (mg h$^{-1}$ cell$^{-1}$)</th>
<th>$R_{Anti}$ (mg h$^{-1}$ cell$^{-1}$)</th>
<th>$S_{Max}$ (mg cell$^{-1}$)</th>
<th>$k$ (Ml$^{-1}$ mg$^{-1}$ h$^{-1}$)</th>
<th>$T_{G1a}$ (h)</th>
<th>$T_S$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.544x10$^{-8}$</td>
<td>1.079x10$^{-8}$</td>
<td>2.588x10$^{-9}$</td>
<td>5.562x10$^{-3}$</td>
<td>2.5</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 4.1 - Model parameters used to simulate the process.

In batch operation, the bias in Equation 4.1 was the initial model parameter value in the copy of the model which resides in the model loop. In repeated batch operation, this value only applies to the first resuspension cycle. At the changeover of cycles, the non-harvested bioreactor contents are diluted according to the value of the harvest fraction (HF). Therefore, the values of the model outputs and the process outputs decrease. This results in a decrease in the term $c_q(t)$ (Equations 4.2 and 4.3) and, consequently, in a decrease in the model parameter value (Equation 4.1). Clearly, the
model parameter should not change due to dilution. The model adaptor algorithm accounts for this situation by resetting the bias at the beginning of each cycle so that the model parameter is the same as at the end of the previous cycle; this is referred to as 'bump-free' operation.

In a real situation, the process outputs would be measurable variables; the process outputs have thus been selected on that basis. They were: the viable cell, glutamine, ammonia and antibody concentrations. The model adaptor compared these outputs to the corresponding model outputs in order to manipulate a model parameter to adapt the model.

4.2.2 Model error and parameter error

Model adaptation results in the model output being driven towards the process output, as explained above. The model error, \( e_m(t) \), is the difference between the process output (the ‘desired’ value) and the model output (see Figure 4.1). The parameter error, \( e_{par}(t) \), is the relative error between the model parameter value and that used to simulate the process. These errors are given by the following expressions:

\[
e_m(t) = y_p(t) - y_m(t) \tag{4.4}
\]

and

\[
e_{par}(t) = \frac{x_m(t) - x_p}{x_p} \tag{4.5}
\]

where \( x_p \) is the model parameter value used to simulate the process (units as appropriate).

Substituting \( x_m(t) \) by Equation 4.1, the previous expression can be re-written as:
\[ e_{par}(t) = \frac{x_b(t)}{x_p} + \frac{c_a(t)}{x_p} - 1 \]  

(4.6)

where \( x_p \) is a constant value; \( x_b(t) \) is constant in batch operation and constant within each resuspension cycle in repeated batch operation; \( c_a(t) \) is defined by Equations 4.2 or 4.3, depending on the type of action employed. Therefore, it can be concluded that the parameter error is proportional to the model error, for proportional-only action and is a linear function of the model error, for proportional-integral action. This is always valid in batch operation; however, in repeated batch, it is only true within each resuspension cycle due to the assumption of ‘bump-free’ operation (Section 4.2.1). Despite this relationship, model and parameter errors are not equal, as shown below.

Equation 4.1 can be re-written, considering proportional-only action, as:

\[ e_m(t) = \frac{1}{K_a} \left[ x_m(t) - x_b(t) \right] \]  

(4.7)

or, for proportional-integral action, as:

\[ e_m(t) + \frac{1}{\tau_a} \int_0^t e_m(t) \, dt = \frac{1}{K_a} \left[ x_m(t) - x_b(t) \right] \]  

(4.8)

When the manipulated model parameter reaches its desired value, i.e., \( x_p \), the model error is not zero; thus, the model output does not reach its desired value. Therefore, it can be concluded that model error and parameter error are not equal.

It should be noted that the proportionality between model and parameter errors shown above cannot be observed when the manipulated model parameters are \( T_{G1a} \) or \( T_s \). This is due to the method employed to solve the model, by which cell cycle phases are divided into an integer number of time elements, referred to as age elements.
Consequently, the model adaptor algorithm cannot attribute non-integer values to $T_{G1a}$ and $T_S$; thus, model and parameter errors are not proportional for these parameters.

### 4.3 Testing of the Model Adaptor Algorithm

The model adaptor algorithm was tested in batch operation, over 150 h, for an initial viable cell concentration of $0.09 \times 10^6$ cell ml$^{-1}$ and an initial glutamine concentration of 5 mg ml$^{-1}$. The inoculum state was that described in Section 3.5.1, i.e., all cells were initially evenly distributed throughout the G1' phase. Both process and model were simulated at these conditions and the model was adapted on-line to 'correct' an initial parameter mismatch; a sampling frequency of 6 min was considered, which corresponds to the step length used for the Euler Integration.

The algorithm was also tested in repeated batch operation; the results obtained will be addressed in Chapter 5. Testing of the algorithm followed the same methodology for either operation mode; thus, the features presented in this section also apply to the investigation conducted in repeated batch operation.

#### 4.3.1 Simulations set up

As stated in Section 4.2.1, six model parameters were chosen to be separately manipulated. In order to test the control algorithm, these parameters were initially changed by $\pm 10\%$ ($\pm 8\%$ for $T_{G1a}$) in the copy of the model which resides in the model loop; in other words, each model parameter in the model was initially 10\% (or 8\%) greater or lower than the value used to simulate the process. The initial mismatch in $T_{G1a}$ was $\pm 8\%$ due to the restriction to integer values imposed to both $T_{G1a}$ and $T_S$, as explained in Section 4.2.2.

The outputs used by the model adaptor to manipulate the selected model parameters were dependent upon the parameter itself. The combinations investigated are
Development and Testing of the Model Adaptor Algorithm

presented in Table 4.2. The unexplored combinations correspond to the cases where the model parameter has no effect on the outputs considered; this is due to the structure of the model.

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Initial mismatch</th>
<th>Measured outputs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cell</td>
</tr>
<tr>
<td>( R_{\text{Glut}} )</td>
<td>-10%</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>+10%</td>
<td>✓</td>
</tr>
<tr>
<td>( R_{\text{Ant}} )</td>
<td>-10%</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>+10%</td>
<td></td>
</tr>
<tr>
<td>( S_{\text{Max}} )</td>
<td>-10%</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>+10%</td>
<td>✓</td>
</tr>
<tr>
<td>( k )</td>
<td>-10%</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>+10%</td>
<td>✓</td>
</tr>
<tr>
<td>( T_{\text{Gla}} )</td>
<td>-8%</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>+8%</td>
<td>✓</td>
</tr>
<tr>
<td>( T_{S} )</td>
<td>-10%</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>+10%</td>
<td>✓</td>
</tr>
</tbody>
</table>

Table 4.2 - Scenarios chosen for testing the model adaptor algorithm. (Cell, Glut, Anti and Ammo are the viable cell, glutamine, antibody and ammonia concentrations, respectively.)

4.3.2 Analysis of model error and parameter error

In principle, monitoring the difference between the process and the model outputs (the model error) should be used to assess the success of the adaptation procedure. However, in this work, there is an additional way to investigate model adaptation; this is the on-line monitoring of the parameter error (Equation 4.5).

Manipulation of model parameters may correspond to alteration of the dynamics and/or the structure of the model, which would result in variations in the model outputs. These variations may be assessed through analysis of the model error.
However, this information is also available through analysis of the parameter error due to the proportionality between these errors, as demonstrated in Section 4.2.2. The advantage of analysing the parameter error is that it also enables understanding how the model itself responds to the adaptation algorithm. However, it should be pointed out that calculation of the parameter error was possible as the process was being simulated. Otherwise, the value of $x_p$ might not be known and adaptation would have to be investigated through analysis of the model error only.

A common approach was considered for analysis of model error and parameter error. Both were analysed in terms of:

- the time required to reach the desired value for the first time, referred to as cross time, $t_c$ (h);
- the time required to reach and remain at the desired value, referred to as settling time, $t_s$ (h);
- the ultimate error value, referred to as $e_u$ (units as appropriate); and
- the maximum error which coincides with an inflexion point, referred to as overshoot, $e_{ov}$ (units as appropriate).

These quantities will be regarded as performance measures. As stated previously (Section 4.2.2), model and parameter errors are proportional, but not equal. Therefore, the values for the performance measures for model error are expected to be different from those obtained for parameter error.

Small values for the performance measures are desirable. The parameter error and the model error may reach their desired values in an oscillatory manner. For practical purposes, the settling time was considered to be the time required for the manipulated model parameter or the model output to reach and remain within $\pm 0.5\%$ of their desired values. It should be noted that the 'settling band' is very narrow due to the process being simulated. The only difference between model and process is the value of the manipulated model parameter. Therefore, it is expected for the model to be a good representation of the process and, thus, for both model outputs and model parameters to reach and remain within $\pm 0.5\%$ of their desired values.
If the oscillations exhibited by model and parameter errors reach but do not remain within the above ‘settling band’, they are said to be ‘unacceptable’; this is due to amplification of model error. In such cases, the overshoot will be considered to be the maximum deviation from the desired value before amplification of errors.

The model adaptor parameters \( (K_a, \tau_a) \) influence the value of the performance measures, as well as the presence and amplitude of oscillations in both model error and parameter error. However, performance measures and oscillations may be affected in a different manner, i.e., small values for the performance measures may be accompanied by unacceptable oscillations. If both model and parameter errors exhibit unacceptable oscillations or irreversibly deviate from their desired values, regardless of the value of the model adaptor parameters, model adaptation is said to be impossible.

The criterion adopted for successful model adaptation was the minimisation of the model error. In order to achieve this, the model adaptor parameters were optimised so that the performance measures were as small as possible, without the presence of ‘unacceptable’ oscillations. Those parameters were designated as optimal \( K_a \) and optimal \( \tau_a \), although the optimisation conducted was not rigorous. It was only necessary to have an estimate of the optimal model adaptor parameters as they may be different when the model adaptor and process controller algorithms are used simultaneously.

### 4.4 Results Obtained in Batch Operation

The process behaviour obtained at the initial conditions considered was presented and discussed earlier (Figure 3.5, Section 3.5.1). It has been observed that glutamine is in excess and that viable cell concentration increases throughout the simulation; no control action was thus required at these conditions. The model adaptor algorithm was tested for the combinations of model parameter/measured output presented in
Table 4.2: greater initial parameter mismatches were also considered for some of
those combinations. Adaptation was conducted on-line, using a sampling frequency
of 6 min; both proportional-only and proportional-integral actions were considered.
A general overview of the results obtained will be presented in Section 4.4.1; some of
these will be shown in more detail in Section 4.4.2.

4.4.1 Overview of results

It has been observed that manipulation of $R_{\text{Glut}}$, $R_{\text{Anti}}$ and $S_{\text{Max}}$ resulted in successful
model adaptation for some of the measured outputs considered. Adaptation proved
impossible for the other model parameters considered, i.e., $k$, $T_{\text{Gla}}$ and $T_{S}$.

Overall, worse results were obtained when considering proportional-integral action
than when employing proportional-only action. Although the introduction of the
integral term was beneficial in terms of cross time and ultimate error, it led to more
oscillations.

The effect of greater initial mismatches was analysed for the model parameters which
were successfully adapted for a $\pm 10\%$ initial mismatch; $\pm 20\%$ and $\pm 50\%$ mismatches
were considered. It was observed that the effect of different initial mismatches was the
same for all the model parameters considered, regardless of the type of action
employed. Furthermore, the results obtained for initial positive mismatches were the
same as, or worse than, those obtained for initial negative mismatches.

4.4.2 Detailed results

Unless otherwise stated, it should be assumed that the results presented below were
obtained considering proportional-only action and $\pm 10\%$ initial mismatches.
4.4.2.1 Manipulation of R\textsubscript{Glut}

Model adaptation by manipulation of R\textsubscript{Glut} was successful when using either viable cell, glutamine or ammonia concentrations as the measured output, but not when using antibody concentration.

The model error and the parameter error obtained when manipulating R\textsubscript{Glut} using viable cell concentration as measured output, employing proportional-only action, are presented in Figures 4.2 and 4.3, respectively. \( K_a \) was varied between \( 6 \times 10^{-16} \) and \( 1.2 \times 10^{-13} \) mg ml\(^{-1}\) cell\(^{-2}\); values greater than \( 4 \times 10^{-14} \) mg ml\(^{-1}\) cell\(^{-2}\) led to unacceptable oscillations. The results presented in Figures 4.2 and 4.3 are representative of the model and parameter errors obtained for the whole range of \( K_a \) values considered.

Cross time could not be defined for model error, as can be seen in Figure 4.2; settling times could not be defined for either model error or parameter error, according to the definition adopted (Section 4.3.2).

It can be seen in Figures 4.2 and 4.3 that an increase in \( K_a \) corresponds to an increase in oscillations and a decrease in ultimate error and overshoot for both model and parameter errors; it also corresponds to a decrease in cross time for parameter error. It may also be noted that the amplitude of the overshoot does not decrease linearly with \( K_a \) and appears to approach an asymptote; this is more perceptible in Figure 4.3. It can also be seen that there is an initial lag in both model and parameter errors, regardless of the value of \( K_a \).

As explained in Section 4.3.2, the optimal \( K_a \) value is that which corresponds to the smallest performance measures, without the presence of unacceptable oscillations. It can be seen in Figures 4.2 and 4.3 that the optimal \( K_a \) value in this case is \( 3 \times 10^{-14} \) mg ml\(^{-1}\) cell\(^{-2}\). The values for the performance measures obtained at this \( K_a \) value, for a -10% initial mismatch, are presented in Table 4.3 (Page 56), for both model and parameter errors.
It can be seen in Figures 4.2 and 4.3 that varying $R_{\text{Glut}}$ by ±10% at the optimal $K_a$ value does not result in 'mirrored' behaviour for either model error or parameter error. The cross time is the same, but the overshoot is greater and is observed later for the initial negative mismatch.

The model error and the parameter error obtained for adaptation of $R_{\text{Glut}}$ using glutamine concentration as measured output are presented in Figures 4.4 and 4.5, respectively. $K_a$ was varied between $-6 \times 10^{-09}$ and $-1.5 \times 10^{-05}$ ml h$^{-1}$ cell$^{-1}$; values greater than $-1 \times 10^{-05}$ ml h$^{-1}$ cell$^{-1}$ led to unacceptable oscillations.

It can be observed that settling time could not be defined for the model error. Overall, the model is adapted more quickly than when viable cell concentration was used as measured output (Figures 4.2 and 4.3), even if the initial lag in the latter is ignored. It may also be noted that the magnitude of the overshoots in Figure 4.5 are smaller than those in Figure 4.3 and do not appear to approach an asymptote. The optimal $K_a$ value was determined to be $-6 \times 10^{-06}$ ml h$^{-1}$ cell$^{-1}$. Varying $R_{\text{Glut}}$ by ±10% at this $K_a$ value resulted in 'mirrored' behaviour for both model and parameter errors; the results obtained for a -10% initial mismatch are summarised in Table 4.3.

The model error and the parameter error for adaptation of $R_{\text{Glut}}$ using ammonia concentration as measured output will not be presented here as they match those obtained using glutamine concentration both qualitatively and quantitatively. The values for the performance measures obtained at the optimal $K_a$ value, for a -10% initial mismatch, are presented in Table 4.3, for both model and parameter errors.

$R_{\text{Glut}}$ was also adapted employing proportional-integral action in order to eliminate the ultimate error. $\tau_a$ was varied at the optimal $K_a$ in order to achieve a compromise between small performance measures and acceptable oscillations and, thus, define an optimal $\tau_a$ value. A brief study of the effect of $K_a$ upon adaptation was also conducted. It has been observed that both model and parameter errors were qualitatively the same as those obtained for proportional-only action, regardless of the measured output used. It has also been observed that an increase in $\tau_a$ has the opposite effect of an increase in
Ka upon the performance measures and oscillations exhibited. The results obtained at the optimal model adaptor parameters \((K_a, \tau_a)\), for an initial -10\% mismatch, are summarised in Table 4.4 (Page 56). In comparison with Table 4.3, it can be observed that cross times and most ultimate errors were smaller. However, larger settling times were observed for the parameter error.

As stated in Section 4.4.1, the effect of greater initial mismatches was the same for all the model parameters considered, whatever the control action employed. The model error and the parameter error observed for adaptation of \(R_{\text{Glu}}\) using glutamine concentration as measured output, for different initial mismatches, are presented as an example in Figures 4.6 and 4.7, respectively. These were obtained at the optimal \(K_a\) value (Table 4.3) and considering proportional-only action. It can be observed that, qualitatively, the variation in parameter error is the same. Quantitatively, greater initial mismatches result in greater overshoot; cross time (defined only for parameter error) and settling time remain the same. It can also be observed that ‘mirrored’ behaviour was only obtained for ±10\% initial mismatches. For initial mismatches of ±20\% and ±50\%, the overshoot is always greater for the initial negative mismatches.

### 4.4.2.2 Manipulation of \(S_{\text{Max}}\)

Model adaptation by manipulation of \(S_{\text{Max}}\) was only successful when using viable cell concentration as measured output. The model error and the parameter error obtained were qualitatively the same as for adaptation of \(R_{\text{Glu}}\) using this measured output (Figures 4.2 and 4.3); the quantitative differences were small. These observations apply both to proportional-only and proportional-integral actions.

The optimal model adaptor parameters were defined in the same manner as for \(R_{\text{Glu}}\). The performance measures for both model and parameter errors obtained at the optimal model adaptor parameters, for a -10\% initial mismatch and for both actions employed, are presented in Table 4.5 (Page 57).
4.4.2.3 Manipulation of $R_{\text{Anti}}$

$R_{\text{Anti}}$ was manipulated using only antibody concentration as measured output (Table 4.2). The model error and the parameter error obtained for adaptation of $R_{\text{Anti}}$ are presented in Figures 4.8 and 4.9, respectively. $K_a$ was varied between $6 \times 10^{-09}$ and $8 \times 10^{-06}$ ml h$^{-1}$ cell$^{-1}$; values greater than $6 \times 10^{-06}$ ml h$^{-1}$ cell$^{-1}$ led to unacceptable oscillations.

It can be seen in Figures 4.8 and 4.9 that there are no overshoots. This resulted in cross time and settling time being the same for the parameter error; neither of these performance measures could be determined for the model error.

It has been observed that an increase in $K_a$ corresponds to a decrease in ultimate error. It also corresponds to a decrease in cross (or settling) time for the parameter error (Figure 4.9). For very large $K_a$ values, the small model error is considerably amplified towards the end of the simulation (Figure 4.8); this is also noticeable in terms of parameter error (Figure 4.9). The optimal $K_a$ value was determined to be $5.4 \times 10^{-06}$ ml h$^{-1}$ cell$^{-1}$. Varying $R_{\text{Anti}}$ by $\pm 10\%$ at this value resulted in 'mirrored' behaviour for both errors; the results obtained for a $-10\%$ initial mismatch, are presented in Table 4.6 (Page 57).

Introduction of an integral term to the model adaptor algorithm was conducted as previously described for manipulation of $R_{\text{Glut}}$ (Section 4.4.2.1). The model error and the parameter error obtained for various $\tau_a$ values are presented in Figures 4.10 and 4.11, respectively; the equivalent errors obtained at the same (optimal) $K_a$ value for proportional-only action are included, for comparison. $\tau_a$ was varied between $8.3 \times 10^{-02}$ and 35 h; no unacceptable oscillations were exhibited. It can be seen that proportional-integral action results in more oscillations than proportional-only action, but also in smaller cross times (Figure 4.11). Settling times could be determined for both errors; for parameter error, these values were larger than those obtained for proportional-only action.
It can be seen that a variation in \( \tau_a \) has a different effect upon the magnitude of the overshoot in model error and parameter error. The optimal \( \tau_a \) value was determined to be 33.3 h. Varying \( R_{\text{Anti}} \) by \( \pm 10\% \) at the optimal \( K_a \) and \( \tau_a \) values resulted in 'mirrored' behaviour for both model and parameter errors. The results obtained for a -10\% initial mismatch are presented in Table 4.6.

### 4.4.2.4 Manipulation of \( k, T_{G1a} \) and \( T_S \)

Manipulation of these model parameters resulted in unsuccessful model adaptation when employing proportional-only action. The results obtained for proportional-integral action were qualitatively the same; quantitatively, the performance measures obtained were worse.

Manipulation of \( k \) resulted in both model and parameter errors exhibiting unacceptable oscillations, regardless of the values for model adaptor parameters. For the adaptation of \( T_{G1a} \) or \( T_S \), it was possible to define cross times for both errors when using viable cell concentration as measured output, but not for the other measured outputs considered. It should be noted that, qualitatively, manipulation of \( T_{G1a} \) and \( T_S \) yielded similar adaptation results; quantitatively, adaptation was more sensitive to changes in \( T_S \).

The model error and the parameter error obtained by manipulation of \( T_{G1a} \) using viable cell concentration as measured output, for proportional-only action, are presented in Figures 4.12 and 4.13, respectively. \( K_a \) was varied between \(-3.8\times10^{-6}\) and \(-4\times10^{-5}\) h ml cell\(^{-1}\); values greater than \(-3\times10^{-5}\) h ml cell\(^{-1}\) led to unacceptable oscillations.

It can be seen in Figure 4.12 that the model error is a continuous function, although \( T_{G1a} \) is manipulated in a step-wise manner (Figure 4.13); settling times cannot be calculated for either error. It can also be seen that an increase in \( K_a \) has the same effect on performance measures and oscillations as when manipulating any of the other model parameters. However, it was not possible to determine an optimal \( K_a \) value.
### Table 4.3 - Batch operation: performance measures obtained at the optimal model adaptor parameters for adaptation of $R_{\text{Glut}}$ considering proportional-only action (-10% initial mismatch).

<table>
<thead>
<tr>
<th>Model adaptor parameters</th>
<th>Viable cell concentration (cell ml$^{-1}$)</th>
<th>Glutamine concentration (mg ml$^{-1}$)</th>
<th>Ammonia concentration (mg ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_a$</td>
<td>$3 \times 10^{-14}$ mg ml$^{-1}$ h$^{-1}$ cell$^{-2}$</td>
<td>$-6 \times 10^{-06}$ ml h$^{-1}$ cell$^{-1}$</td>
<td>$6 \times 10^{-05}$ ml h$^{-1}$ cell$^{-1}$</td>
</tr>
<tr>
<td>$e_u$</td>
<td>$0.069 \times 10^{-06}$ cell ml$^{-1}$</td>
<td>$-0.26 \times 10^{-03}$ mg ml$^{-1}$</td>
<td>$0.026 \times 10^{-02}$ mg ml$^{-1}$</td>
</tr>
<tr>
<td>$e_{ov}$</td>
<td>$0.112 \times 10^{-06}$ cell ml$^{-1}$</td>
<td>$-0.33$ mg ml$^{-1}$</td>
<td>$-0.035$ mg ml$^{-1}$</td>
</tr>
<tr>
<td>$e_x$</td>
<td>$59$ h</td>
<td>$18$ h</td>
<td>$17$ h</td>
</tr>
<tr>
<td>$e_v$</td>
<td>$58$ h</td>
<td>$55$ h</td>
<td>$55$ h</td>
</tr>
<tr>
<td>$e_{uv}$</td>
<td>$3.3%$</td>
<td>$0.003%$</td>
<td>$0.003%$</td>
</tr>
<tr>
<td>$e_{uv'}$</td>
<td>$11.8%$</td>
<td>$3.7%$</td>
<td>$3.7%$</td>
</tr>
</tbody>
</table>

$^1$ no value could be determined for $t_x$ or $t_v$.

$^*t$ no value could be determined.

### Table 4.4 - Batch operation: performance measures obtained at the optimal model adaptor parameters for adaptation of $R_{\text{Glut}}$ considering proportional-integral action (-10% initial mismatch).

<table>
<thead>
<tr>
<th>Model adaptor parameters</th>
<th>Viable cell concentration (cell ml$^{-1}$)</th>
<th>Glutamine concentration (mg ml$^{-1}$)</th>
<th>Ammonia concentration (mg ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_a$</td>
<td>$3 \times 10^{-14}$ mg ml$^{-1}$ h$^{-1}$ cell$^{-2}$</td>
<td>$-6 \times 10^{-06}$ ml h$^{-1}$ cell$^{-1}$</td>
<td>$6 \times 10^{-05}$ ml h$^{-1}$ cell$^{-1}$</td>
</tr>
<tr>
<td>$t_s$</td>
<td>$33.3$ h</td>
<td>$8.3$ h</td>
<td>$8.3$ h</td>
</tr>
<tr>
<td>$e_u$</td>
<td>$0.072 \times 10^{-06}$ cell ml$^{-1}$</td>
<td>$-0.24 \times 10^{-03}$ mg ml$^{-1}$</td>
<td>$0.025 \times 10^{-02}$ mg ml$^{-1}$</td>
</tr>
<tr>
<td>$e_{ov}$</td>
<td>$0.130 \times 10^{-06}$ cell ml$^{-1}$</td>
<td>$-0.4$ mg ml$^{-1}$</td>
<td>$-0.05$ mg ml$^{-1}$</td>
</tr>
<tr>
<td>$t_x$</td>
<td>$52$ h</td>
<td>$14$ h</td>
<td>$13$ h</td>
</tr>
<tr>
<td>$t_v$</td>
<td>$65$ h</td>
<td>$62$ h</td>
<td>$62$ h</td>
</tr>
<tr>
<td>$e_x$</td>
<td>$4.0%$</td>
<td>$0.002%$</td>
<td>$0.003%$</td>
</tr>
<tr>
<td>$e_{uv}$</td>
<td>$12.0%$</td>
<td>$5.0%$</td>
<td>$5.2%$</td>
</tr>
</tbody>
</table>

$^1$ no value could be determined for $t_x$.

$^*t$ no value could be determined.

---

$t_x$ - cross time: time required to reach the desired value for the first time (h).

t_v - settling time: time required to reach and remain at the desired value (h).

e_u - ultimate error value (units as appropriate).

e_{uv} - overshoot: maximum error which coincides with an inflection point (units as appropriate).
## Development and Testing of the Model Adaptor Algorithm

### Viable cell concentration (cell ml⁻¹)

<table>
<thead>
<tr>
<th>Model adaptor parameters</th>
<th>( K_a )</th>
<th>( \tau_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-7\times10^{-13}) mg ml⁻¹ cell⁻²</td>
<td>33.3 h</td>
</tr>
</tbody>
</table>

### Measured output

<table>
<thead>
<tr>
<th>Performance measures††</th>
<th>( e_u )</th>
<th>( e_{ov} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-0.040\times10^{-06}) cell ml⁻¹</td>
<td>(-0.039\times10^{-06}) cell ml⁻¹</td>
</tr>
<tr>
<td></td>
<td>( 0.092\times10^{-06}) cell ml⁻¹</td>
<td>(-0.101\times10^{-06}) cell ml⁻¹</td>
</tr>
</tbody>
</table>

### Parameters

<table>
<thead>
<tr>
<th>Performance measures listing</th>
<th>( t_c )</th>
<th>( t_s )</th>
<th>( e_u )</th>
<th>( e_{ov} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-0.040\times10^{-06}) cell ml⁻¹</td>
<td>51 h</td>
<td>46 h</td>
<td>5.0 %</td>
<td>12.5 %</td>
</tr>
<tr>
<td>(-0.039\times10^{-06}) cell ml⁻¹</td>
<td>(-0.101\times10^{-06}) cell ml⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4.5 - Batch operation: performance measures* obtained at the optimal model adaptor parameters for adaptation of \( S_{max} \) considering both proportional-only and proportional-integral actions (-10% initial mismatch).

### Antibody concentration (mg ml⁻¹)

<table>
<thead>
<tr>
<th>Model adaptor parameters</th>
<th>( K_a )</th>
<th>( \tau_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( 5.4\times10^{-06}) ml⁻¹ h⁻¹ cell⁻¹</td>
<td>33.3 h</td>
</tr>
</tbody>
</table>

### Measured output

<table>
<thead>
<tr>
<th>Performance measures††</th>
<th>( e_u )</th>
<th>( e_{ov} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( 0.20\times10^{-04}) mg ml⁻¹</td>
<td>( 2.5\times10^{-06}) mg ml⁻¹</td>
</tr>
</tbody>
</table>

### Parameters

<table>
<thead>
<tr>
<th>Performance measures listing</th>
<th>( t_c )</th>
<th>( t_s )</th>
<th>( e_u )</th>
<th>( e_{ov} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 0.20\times10^{-04}) mg ml⁻¹</td>
<td>20 h</td>
<td>14 h</td>
<td>0 %</td>
<td>( 20 h )</td>
</tr>
<tr>
<td>( 2.5\times10^{-06}) mg ml⁻¹</td>
<td>(-0.040\times10^{-06}) cell ml⁻¹</td>
<td>(-0.039\times10^{-06}) cell ml⁻¹</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4.6 - Batch operation: performance measures* obtained at the optimal model adaptor parameters for adaptation of \( R_{Ant} \) considering both proportional-only and proportional-integral actions (-10% initial mismatch).

---

* \( t_c \) - cross time: time required to reach the desired value for the first time (h).
* \( t_s \) - settling time: time required to reach and remain at the desired value (h).
* \( e_u \) - ultimate error value (units as appropriate).
* \( e_{ov} \) - overshoot: maximum error which coincides with an inflexion point (units as appropriate).
It can also be seen in Figures 4.12 and 4.13 that varying $T_{G1a}$ by ±8% at a $K_a$ value of $-1.7 \times 10^{-65}$ h ml cell$^{-1}$ led to 'mirrored' behaviour for both model and parameter errors.

### 4.5 Discussion of Results

It is clear from the results presented in Section 4.4 that the model could be adapted in batch operation for some model parameter/measured output combinations. The aim of this section is to analyse and discuss all results obtained; discussion will focus on the influence upon adaptation of both model adaptor parameters and model properties.

#### 4.5.1 Effect of model adaptor parameters

It has been observed that the model adaptor parameters ($K_a$, $\tau_a$) influence the performance measures of both model and parameter errors and, thus, the success of model adaptation. An increase in $K_a$ corresponded to an increase in oscillations and to a decrease in cross time, settling time and ultimate error for both model error and parameter error; the same effect was observed when decreasing $\tau_a$. The only exception was observed when adapting $R_{Ami}$ as no oscillations were exhibited by either model or parameter errors. In this case, an increase in $K_a$ (or a decrease in $\tau_a$) corresponded to a decrease in settling (and cross) time.

The above observations correspond to those expected of the response of a linear system to proportional-only and proportional-integral actions (Stephanopoulos, 1985; Seborg et al., 1989). However, it has also been observed that an increase in $K_a$ (or a decrease in $\tau_a$) corresponds to a decrease in overshoot (when present). This contradicts conventional linear theory (Stephanopoulos, 1985; Seborg et al., 1989) and may be indicative of non-linear behaviour. This would be expected of the model used due to its conceptual features (Section 3.4.2), namely the random transitions between phases. Therefore, further analysis of the results obtained with respect to the properties of the model is of vital importance.
The advantage of proportional-integral action over proportional-only action is the elimination of the ultimate error, characteristic of the latter, although it results in more oscillations (Stephanopoulos, 1985; Seborg et al., 1989). Overall, it has been observed that the introduction of an integral term to the model adaptor action was beneficial in terms of cross time and ultimate error, but not in terms of settling time or degree of oscillations. Performance measures similar to those obtained when employing proportional-only action were accompanied by unacceptable oscillations in both model and parameter errors, for some of the combinations explored. Furthermore, the optimal $\tau_a$ values determined when model adaptation was successful were, in general, extremely large. Therefore, the integral term has little weight on the overall action term (Equation 4.3). These observations indicate that, overall, there is no advantage in introducing an integral term to the model adaptor action. This is due to the fact that the ultimate values for both model and parameter errors were already very small for proportional-only action (Tables 4.3, 4.5 and 4.6) and oscillations were of small magnitude.

It has been observed that model adaptation was not successful for all explored combinations; this was due to amplification of small model errors when increasing $K_a$ (or decreasing $\tau_a$). The small errors occurred as a result of the interdependency between the manipulated model parameters and the measured outputs. For example, adaptation of $R_{Glut}$ using antibody concentration proved impossible. The value of $R_{Glut}$ determines the consumption of glutamine (Section 3.4.2), thus affecting the growth rate. However, varying $R_{Glut}$ results in small changes in viable cell concentration which, in turn, result in small changes in antibody concentration and, thus, in small model error. Increasing $K_a$ (or decreasing $\tau_a$) in order to decrease the value of the performance measures resulted in both model and parameter errors exhibiting unacceptable oscillations.

**4.5.2 Effect of model properties**

Qualitative similarities were observed between some of the adaptation results
obtained. This was due to some model parameters having similar effects upon the selected model outputs. For example, similarities were observed between the results obtained for $R_{\text{Glut}}$ and $S_{\text{Max}}$ using viable cell concentration as measured output. This was due to an increase in $R_{\text{Glut}}$ having a similar effect upon growth rate and, thus, viable cell concentration as a decrease in $S_{\text{Max}}$ (Section 3.4.2); the opposite variations in the model parameters were reflected by the positive $K_a$ values for $R_{\text{Glut}}$ and negative values for $S_{\text{Max}}$. Similar adaptation results were also obtained when manipulating $T_{G1\alpha}$ and $T_S$ using any of the selected measured outputs. This was due to both parameters affecting cell cycle time. It has been observed that adaptation was quantitatively more sensitive to changes in $T_S$ than in $T_{G1\alpha}$. This was due to $T_S$ being greater than $T_{G1\alpha}$, which resulted in greater initial mismatch. Consequently, the difference in cell cycle time relative to that of the process was greater and, thus, model errors were greater.

It has also been observed that using either glutamine or ammonia concentrations as measured output, for the same manipulated model parameter, yielded adaptation results which were qualitatively similar. This was due to production of ammonia being a direct result of glutamine metabolism (Section 3.4.2) and was thus expected.

Manipulation of $T_{G1\alpha}$ and $T_S$ resulted in unsuccessful adaptation, regardless of the measured output considered, due to amplification of model errors, as discussed in Section 4.5.1. It has also been observed that model and parameter errors were not proportional when adapting either of these parameters, as expected (Section 4.2.2). Varying these parameters requires changing the number of age elements in phases $G1\alpha$ and $S$ and, thus, affects the average cell cycle time; this may be referred to as structural mismatch (Section 2.4.3). Changing the number of age elements in either of these phases results in a small change in cell age distribution. Consequently, cells have to be re-distributed in these phases so that both the total number of cells in these phases and the relative percentage of cells in each of the age elements are maintained. The parameter error is discontinuous (see Figure 4.13) as the number of age elements are changed in integer steps, as stated in Section 4.2.2.
4.5.3 Cell age distribution

Analysis of cell age distribution allows a more detailed understanding of the underlying dynamics of the model. The distributions obtained by the model for adaptation of $R_{\text{Glut}}$ using viable cell concentration as measured output will be discussed below, as an example of the analysis conducted. The effect of varying $R_{\text{Glut}}$ on the model outputs was detailed previously (Section 4.5.1). It may also be noted that changes in glutamine concentration result in changes in the cumulative amount of glutamine of $G1b$ cells. This directly influences the $G1b/S$ transition (Section 3.4.2) and, thus, the growth rate.

It has been observed that there is an initial lag in both model and parameter errors for this model parameter/measured output combination (Figures 4.2 and 4.3). This was expected as the initial cell age distribution was chosen so as to simulate the initial lag period of growth curves. Cells initially in $G1'$ progressively enter the cell cycle via $G1b$ (Section 3.4.2). Those $G1b$ cells which immediately enter $S$ via the random transition take 9 h to reach the end of $M$ and undergo cytokinesis; this time interval corresponds to the durations of phases $S$, $G2$ and $M$ (Section 3.4.2). Therefore, there is no change in viable cell concentration during this lag period; there is no model error and, thus, $R_{\text{Glut}}$ cannot be adapted.

An extract of the cell age distributions obtained at the optimal $K_a$ value (Table 4.3) for the ±10% initial mismatches and that for the process are presented in Figure 4.14. It can be seen that, after the initial lag period, different cell age distributions were obtained for the different initial parameter mismatches. This was expected as initial mismatches of -10% and +10% have opposite effects on glutamine concentration and, thus, on growth rate. However, it can be observed that the initial mismatches do not affect cell age distribution by the same amount. These different deviations from the process distribution are due to the initial mismatches affecting cell age distribution to a different extent and arise at the beginning of the simulation.
The distributions obtained for both model and process for the initial 20 h are presented in Figure 4.15. It can be observed that a -10% initial mismatch results in cells remaining in G1b for longer than for the process; this is due to glutamine being initially consumed at a lower rate. The rate of transition from G1b to S is thus lower as it is dependent on the cumulative glutamine content of cells (Section 3.4.2). Consequently, more cells enter the D phase, subsequently dying. It can also be seen in Figure 4.15 that the opposite situation occurs for a +10% initial mismatch, resulting in less cells entering the D phase. Therefore, overall, there will be more cells in the cell cycle which results in greater model errors and, thus, in the system being able to respond more quickly to changes in RGlut. This was the reason for the subsequent different deviations from the process distribution for the different initial parameter values. These different deviations corresponded to the overshoot for both model and parameter error being smaller and observed earlier for the +10% initial mismatch (Figures 4.2 and 4.3); thus both errors exhibited 'non-mirrored' behaviour.

It has also been observed that greater initial mismatches resulted in greater response speeds and in greater overshoots (when present) for both model and parameter errors, for the cases considered. This is due to greater differences between the cell age distributions for the model and that of the process, which corresponded to greater model errors.

It should be noted that the initial lag in model and parameter errors analysed above for adaptation of RGlut was observed every time viable cell concentration was used as measured output. It can be seen in Tables 4.3 to 4.5 that this lag resulted in large cross time, overshoot and ultimate error, and settling time could not be defined. It can thus be concluded that viable cell concentration is not a useful measured output for model adaptation. It can also be concluded that SMax should not be selected as manipulated model parameter as it could only be adapted when using viable cell concentration as measured output (Table 4.5).
4.6 Concluding Comments

It has been shown that the model adaptor algorithm is capable of adapting the model in batch operation when applying proportional-only action. Introduction of an integral term to the model adaptor action did not prove advantageous as it introduced oscillations to both model and parameter errors, without significantly reducing ultimate error. The effect of the model adaptor parameters upon model and parameter errors was that expected of a linear system; the only exception was the effect upon overshoot (when present).

The adaptation results obtained were successfully explained in terms of the model properties. Furthermore, the use of cell age distribution as a means of understanding the underlying dynamics of the model has been demonstrated. The qualitative similarities observed between some of the adaptation results obtained were explained in terms of the interdependency between model parameters and measured outputs and also between measured outputs. It has been observed that initial negative mismatches may result in different model and parameter errors relative to those obtained with initial positive mismatches due to those interdependencies. It has also been concluded that, for the successful combinations, the initial mismatch could be increased to ±50% without significantly affecting model adaptation.

In conclusion, $k$, $T_{G1a}$ and $T_S$ could not be adapted, regardless of the measured output used. The model parameters for which model adaptation was successful were $R_{Glut}$, $S_{Max}$ and $R_{Anti}$, although not for all combinations explored. It has been concluded that $S_{Max}$ should not be selected as manipulated model parameter as it could only be adapted when using viable cell concentration as measured output, which led to large values for the performance measures. Overall, the best adaptation results were obtained by manipulation of $R_{Anti}$ using antibody concentration as measured output.

In general, adaptation was achieved with large settling times, except when manipulating $R_{Anti}$; this may be undesirable in an experimental situation. The impact
of this limitation may be considerably reduced if the process is operated for a longer period of time or in repeated batch. The results obtained when further testing the model adaptor algorithm in repeated batch operation will be presented and discussed in the next chapter.
Chapter 5
Further Testing of the
Model Adaptor Algorithm

5.1 Introduction

The development of the model adaptor algorithm and its testing in batch operation have been addressed in Chapter 4. It has been shown that the algorithm was capable of adapting the model for the majority of the model parameter/ measured output combinations explored. However, large settling times were generally observed. The impact of this observation on model adaptation may be reduced if the process is operated in repeated batch.

In this chapter, the testing of the model adaptor algorithm in repeated batch operation will be addressed. This testing followed the methodology outlined in Section 4.3, for batch operation. In addition, the effect upon model adaptation of modifying model adaptor action parameters was also investigated. The action parameters addressed were sampling frequency, sampling noise and hidden mismatches.

5.2 Testing the Algorithm in Repeated Batch Operation

The model adaptor algorithm was further tested in repeated batch operation. The methodology employed was the same as when considering batch operation and has been detailed in Section 4.3. The algorithm was tested in repeated batch operation using an initial viable cell concentration of $0.09 \times 10^{06}$ cell ml$^{-1}$ and an initial glutamine concentration of 0.1 mg ml$^{-1}$, at a harvest fraction (HF) of 80% and a
Further Testing of the Model Adaptor Algorithm

dilution cycle time (DCT) of 50 h, over 20 resuspension cycles; cells were initially evenly distributed throughout the G1' phase.

The process behaviour obtained at the above conditions was presented and discussed earlier in this dissertation (Figure 3.7, Section 3.5.2). It has been observed that these conditions resulted in periodic behaviour, with minimal variation in final viable cell concentration between consecutive resuspension cycles. Therefore, no control was required whilst adapting the model on-line; a sampling frequency of 6 min was used. All model parameter/measured output combinations presented in Table 4.2 were considered. The model adaptor algorithm was tested employing proportional-only and proportional-integral actions; greater initial parameter mismatches were also considered. Unless otherwise stated, it should be assumed that the results presented below were obtained employing proportional-only action and considering ±10% initial mismatches.

5.3 Results Obtained in Repeated Batch Operation

Model adaptation by manipulation of $R_{\text{Glut}}$ was successful when using either viable cell, glutamine or ammonia concentrations as the measured output, but not when using antibody concentration.

An extract of the model error and the parameter error obtained when manipulating $R_{\text{Glut}}$ using viable cell concentration as measured output are presented in Figures 5.1 and 5.2, respectively; these extracts are representative of the rest of the 20 resuspension cycles. $K_a$ was varied between $3 \times 10^{-14}$ and $3 \times 10^{-12}$ mg ml h$^{-1}$ cell$^{-2}$; values greater than $4 \times 10^{-13}$ mg ml h$^{-1}$ cell$^{-2}$ led to unacceptable oscillations. The results presented in Figures 5.1 and 5.2 are representative of the model and parameter errors obtained for the whole range of $K_a$ values considered. It can be seen that model and parameter errors were only proportional within each resuspension cycle; this was also observed for all other combinations explored.
Further Testing of the Model Adaptor Algorithm

It can be seen in Figures 5.1 and 5.2 that an increase in $K_a$ corresponds to an increase in oscillations and to a decrease in cross time, settling time, ultimate error and overshoot. However, for parameter error, the overshoot increases with $K_a$ when unacceptable oscillations are exhibited. It can also be seen that there is an initial lag in both model and parameter errors, regardless of $K_a$.

The optimal $K_a$ value was determined to be $3 \times 10^{-13}$ mg ml h$^{-1}$ cell$^{-2}$. Varying $R_{\text{Glut}}$ by $\pm 10\%$ at this $K_a$ value resulted in 'non-mirrored' behaviour for both errors. The value for the performance measures obtained at the optimal $K_a$, for a -10% initial mismatch, are presented in Table 5.1 (Page 68).

An extract of the model error and the parameter error obtained by manipulation of $R_{\text{Glut}}$ using glutamine concentration as measured output are presented in Figures 5.3 and 5.4, respectively. $K_a$ was varied between $-6 \times 10^{-06}$ and $-1.5 \times 10^{-04}$ ml h$^{-1}$ cell$^{-1}$; values greater than $-1.3 \times 10^{-04}$ ml h$^{-1}$ cell$^{-1}$ led to unacceptable oscillations.

It can be seen that an increase in $K_a$ has the same effect as when using viable cell concentration as measured output (Figures 5.1 and 5.2). The optimal $K_a$ value was determined to be $-1.2 \times 10^{-04}$ ml h$^{-1}$ cell$^{-1}$. Varying $R_{\text{Glut}}$ by $\pm 10\%$ at the optimal $K_a$ value resulted in 'mirrored' behaviour for both model and parameter errors. The results obtained at the optimal $K_a$, for a -10% initial mismatch, are summarised in Table 5.1.

The model error and parameter error obtained using ammonia concentration as measured output are not presented here as they match those obtained for glutamine concentration, both qualitatively and quantitatively. The results obtained at the optimal $K_a$ value, for a -10% initial mismatch, are presented in Table 5.1.

Adaptation of $S_{\text{Max}}$ was only successful when using viable cell concentration as measured output. Both model and parameter errors were qualitatively the same as for adaptation of $R_{\text{Glut}}$ using the same measured output (Figures 5.1 and 5.2). The results obtained at the optimal $K_a$ value, for a -10% initial mismatch, are summarised in
Further Testing of the Model Adaptor Algorithm

<table>
<thead>
<tr>
<th>Model adaptor parameters</th>
<th>Measured output</th>
<th>Glutamine concentration (mg ml⁻¹)</th>
<th>Ammonia concentration (mg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Viable cell concentration (cell ml⁻¹)</td>
<td>-1.2x10⁻⁰⁴ ml h⁻¹ cell⁻¹</td>
<td>1.2x10⁻⁰⁹ ml h⁻¹ cell⁻¹</td>
</tr>
</tbody>
</table>

### Model Adaptor Performance

<table>
<thead>
<tr>
<th>Performance measures</th>
<th>tₓ</th>
<th>eₓ</th>
<th>eₒₓ</th>
</tr>
</thead>
<tbody>
<tr>
<td>82 h</td>
<td>150 h</td>
<td>148 h</td>
<td></td>
</tr>
<tr>
<td>📌 100%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

### Parameter Adaptor Performance

<table>
<thead>
<tr>
<th>Performance measures</th>
<th>tₓ</th>
<th>eₓ</th>
<th>eₒₓ</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.3 h</td>
<td>10.8 h</td>
<td>10 h</td>
<td></td>
</tr>
<tr>
<td>📌 100%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>- Model parameters</th>
<th>Kₛ</th>
<th>Smax,final</th>
<th>Cmax,final</th>
</tr>
</thead>
<tbody>
<tr>
<td>5x10⁻¹² mg ml⁻¹ cell⁻²</td>
<td>-0.016 cell ml⁻¹</td>
<td>7.2x10⁻⁰⁷ cell ml⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

### Error Measures

<table>
<thead>
<tr>
<th>Error measure</th>
<th>tₓ</th>
<th>eₓ</th>
<th>eₒₓ</th>
</tr>
</thead>
<tbody>
<tr>
<td>📌 -0.016 cell ml⁻¹</td>
<td>7.2x10⁻⁰⁷ cell ml⁻¹</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.1 - Repeated batch operation: performance measures obtained at the optimal model adaptor parameters for adaptation of R_Cl, considering proportional-only action (-10% initial mismatch).

<table>
<thead>
<tr>
<th>Error measure</th>
<th>tₓ</th>
<th>eₓ</th>
<th>eₒₓ</th>
</tr>
</thead>
<tbody>
<tr>
<td>📌 505 h</td>
<td>0%</td>
<td>10%</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2 - Repeated batch operation: performance measures obtained at the optimal model adaptor parameters for adaptation of S_M, considering proportional-only action (-10% initial mismatch).

- tₓ - cross time: time required to reach the desired value for the first time (h).
- tᵧ - settling time: time required to reach and remain at the desired value (h).
- eₓ - ultimate error value (units as appropriate).
- eₒₓ - overshoot: maximum error which coincides with an inflexion point (units as appropriate).
Further Testing of the Model Adaptor Algorithm

<table>
<thead>
<tr>
<th>Model adaptor parameters</th>
<th>Measured output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K_a</td>
</tr>
<tr>
<td></td>
<td>6x10⁻⁶ mg ml⁻¹</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Performance measures</th>
<th>T</th>
<th>C</th>
<th>E</th>
<th>E_U</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₁</td>
<td>105 h</td>
<td>4.8 h</td>
<td>1.8x10⁻⁶ mg ml⁻¹</td>
<td></td>
</tr>
<tr>
<td>ε_u</td>
<td>0 mg ml⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>εₜ</td>
<td>t₁ - t₀</td>
<td>tₙ - t₀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>εₜ</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.3 - Repeated batch operation: performance measures obtained at the optimal model adaptor parameters for adaptation of R_Anti, considering proportional-only action (-10% initial mismatch).

<table>
<thead>
<tr>
<th>Model adaptor parameters</th>
<th>Measured output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K_a</td>
</tr>
<tr>
<td></td>
<td>-3x10⁻⁶ mg ml⁻⁵</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Performance measures</th>
<th>T</th>
<th>C</th>
<th>E</th>
<th>E_U</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₁</td>
<td>346 h</td>
<td>72.7 h</td>
<td>0 cell ml⁻¹</td>
<td></td>
</tr>
<tr>
<td>ε_u</td>
<td>0 cell ml⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>εₜ</td>
<td>t₁ - t₀</td>
<td>tₙ - t₀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>εₜ</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.4 - Repeated batch operation: performance measures obtained at the optimal model adaptor parameters for adaptation of k, considering proportional-only action (-10% initial mismatch).

**Notes:**
- t₁ - cross time: time required to reach the desired value for the first time (h).
- t₀ - settling time: time required to reach and remain at the desired value (h).
- εₜ - ultimate error value (units as appropriate).
- εₜ - overshoot: maximum error which coincides with an inflexion point (units as appropriate).
Further Testing of the Model Adaptor Algorithm

Table 5.2 (Page 68). These performance measures are very similar to those obtained when adapting $R_{\text{Glut}}$ using the same measured output (Table 5.1), although the ultimate errors were not negligible when adapting $S_{\text{Max}}$.

The model error and the parameter error obtained for adaptation of $R_{\text{Anti}}$ using antibody concentration as measured output exhibited no oscillations, regardless of $K_{a}$. Therefore, cross time and settling time were the same values both for model error and parameter error. Similarly, no oscillations were observed for adaptation of $k$ when using viable cell concentration as measured output. The values for the performance measures obtained for both adaptation of $R_{\text{Anti}}$ and $k$, at the optimal $K_{a}$ values, for -10% initial mismatches, are presented in Tables 5.3 and 5.4 (Page 69), respectively.

Manipulation of $T_{\text{Gla}}$ and $T_{S}$ resulted in unsuccessful model adaptation. However, it should be noted that settling times could be determined when manipulating $T_{\text{Gla}}$, although these were very high values ($\approx 600$ h, i.e., 12 cycles).

Overall, introduction of an integral term to the model adaptor action yielded worse results than when proportional-only action was employed; these results will not be presented in detail. Model and parameter errors exhibited similar settling times and smaller cross times, but more oscillations. Furthermore, adaptation proved impossible for some model parameters when proportional-integral action was employed.

The effect of greater initial parameter mismatches was analysed for both action types considered. It was observed that greater initial mismatches resulted in greater response speeds and in greater amplitude of oscillations (when present). The effect was thus the same as in batch operation and detailed results will hence not be presented here.

5.4 Discussion of Results

It is clear from the results presented above that the model has been successfully
adapted in repeated batch operation for the same model parameter/measured output combinations which were successful in batch operation (Section 4.4) and also when manipulating $k$.

Overall, analysis of the adaptation results obtained in repeated batch is qualitatively the same as that conducted for batch operation (Section 4.5). It may be noted that if other HF/DCT values were chosen, the process could exhibit catastrophic failure due to exhaustion of glutamine; thus, the adaptation results would also be quantitatively similar to those obtained in batch operation. However, in this work, the values of HF and/or DCT were not changed as the purpose of this testing was to analyse the model adaptor action; it was assumed that the process controller would control the process effectively, if necessary.

Comparing Tables 5.1 to 5.4 and Tables 4.3 to 4.6, it can be observed that cross time, settling time and overshoot were smaller in repeated batch than in batch operation. The ultimate error was negligible in all cases, except for adaptation of $S_{\text{Max}}$ using viable cell concentration as measured output (Table 5.2), although this error was very small compared to that obtained in batch operation (Table 4.5). It has also been observed that model adaptation was achieved in less than 100 h for the majority of the combinations explored, whereas only $R_{\text{Ami}}$ had been adapted in such a short period in batch operation. It can thus be concluded that the performance measures were better in repeated batch than in batch operation. This was due to being possible to increase $K_a$ to higher values in repeated batch operation as a result of the changes in dynamics introduced through culture removal and medium addition at the changeover of cycles. The dilution of the bioreactor contents at the changeover of cycles was also the reason for the discontinuities exhibited by model error. No discontinuities were observed in parameter error due to the assumption of 'bump-free' operation for the model parameter value (Section 4.2.1).

Manipulation of $k$ using viable cell concentration as measured output resulted in successful model adaptation, which had not been possible in batch operation; this was also due to culture resuspension. The dilution of the bioreactor contents at the end of
each resuspension cycle causes a decrease in viable cell concentration and in ammonia concentration in the medium. Therefore, less cells die than if no resuspension occurred, which leads to greater model errors. Culture resuspension thus resulted in \( k \) affecting viable cell concentration to a greater extent than in batch operation. Consequently, \( K_a \) could be increased to high values without either model or parameter errors exhibiting unacceptable oscillations.

It may be concluded from the adaptation results obtained in repeated batch operation that it is possible to adapt the model in consecutive batches. In fact, all the conclusions previously drawn for model adaptation in batch operation (Section 4.5) could also be drawn in qualitative terms from the results obtained in repeated batch. Therefore, further testing of the model adaptor algorithm was only conducted in repeated batch operation; the results obtained will be presented and discussed in the next section.

## 5.5 Further Investigation in Repeated Batch Operation

The model adaptor algorithm was further investigated in repeated batch to assess whether model adaptation was still possible considering more realistic features. This testing involved less frequent sampling of process outputs, introducing sampling noise to process outputs and introducing hidden mismatches. These features may be referred to as model adaptor action parameters as they affect the way the adaptor manipulates model parameters, i.e., the way the adaptor acts on the model.

### 5.5.1 Simulations set up

The model adaptation results presented in Section 5.3 were obtained considering a sampling frequency of 6 min, i.e., the step length used for the Euler Integration technique (Section 3.4.2). However, it should be noted that measuring viable cell, glutamine, antibody or ammonia concentrations every 6 min is not feasible. In order to
investigate the effect of sampling frequency on model adaptation, process outputs were considered to be sampled at intervals of 0.5, 1, 1.5 and 2 h. Consequently, the model adaptor only manipulated model parameters at these sampling times.

The Box-Müller method was applied to introduce normally distributed (or Gaussian) noise to process outputs in order to simulate real sampling noise. This method consists of generating two independent, normally distributed values, $a$ and $b$, from two independent, uniformly distributed values, $u_1$ and $u_2$ (Morgan, 1990; Marriott, 1997). The normal distribution exhibits mean 0 and variance 1; the uniform distribution generates numbers uniformly distributed between 0 and 1. The normally distributed values are calculated as follows:

\[ a = \sqrt{-2 \ln(u_1)} \cos(2\pi u_2) \]  
\[ b = \sqrt{-2 \ln(u_1)} \sin(2\pi u_2) \]  

Noise levels of 1, 5 and 10% were introduced. A random number generator was added to the model adaptor algorithm to generate numbers uniformly distributed across multiples of the interval $[0,1]$; different noise levels correspond to different multiples of this interval. Introduction of sampling noise means that the process outputs, which are compared to the model outputs in order to adapt the model, have an error associated with their sampling.

An additional parameter mismatch has also been considered to further investigate the model adaptor algorithm; it is referred to as 'hidden' as the algorithm does not manipulate this model parameter. The aim of this testing was to investigate whether the model adaptor action would be affected by an additional, hidden parameter mismatch. The mismatches introduced were related to cell duration and, thus, were directly related to the structure of the model. Three hidden mismatches were separately considered. These were: alteration of the duration of phase $G_{1a}$, $T_{G1a}$; alteration of the duration of phase $S$, $T_S$; and alteration of the flow of cells at the end
of the S phase. $T_{G1a}$ and $T_S$ were altered by $\pm 4\%$ and $\pm 100\%$; a mismatch of $-100\%$ corresponds to removing the phase from the cell cycle and a mismatch of $+100\%$, to doubling its duration. The alteration of cell flow consisted of, at the end of the S phase, 50% of the cells proceeding to G2 and 50% of cells by-passing G2 and immediately entering M. Manipulation of either $T_{G1a}$ or $T_S$ was not considered when introducing any of the above hidden mismatches.

The model adaptor algorithm was tested considering each of the above action parameters separately and also in combination; both proportional-only and proportional-integral actions were employed.

### 5.5.2 Results

A qualitative match was observed between the results obtained when employing proportional-only and proportional-integral actions; quantitatively, those obtained for proportional-integral action were worse. This had already been observed in the main results (Section 5.3); thus, these results will not be presented here. Initial parameter mismatches of $\pm 10\%$, $\pm 20\%$ and $\pm 50\%$ were considered. The effect of greater initial mismatches and of negative or positive mismatches upon both model and parameter errors was also the same as that observed previously. Furthermore, qualitative similarities were still observed between results obtained by manipulation of $R_{Glut}$ and $S_{Max}$, manipulation of $T_{G1a}$ and $T_S$ and using glutamine concentration and ammonia concentration as the measured output.

#### 5.5.2.1 Decreasing sampling frequency

Overall, the effect of sampling frequency on model adaptation was very small, even when process outputs were ‘sampled’ every 2 h. For most manipulated model parameters, negligible differences were observed between the model and parameter errors obtained with 6 min sampling intervals and those obtained with 2 h intervals.
Further Testing of the Model Adaptor Algorithm

It should be noted that ultimate error and settling time were the same but, in some cases, the cross time was smaller and the overshoot greater for lower sampling frequencies.

Manipulation of either $R_{\text{Glut}}$ or $S_{\text{Max}}$ using viable cell concentration as measured output was conducted at the optimal $K_a$ values (Tables 5.1 and 5.2). A decrease in $K_a$ of up to two orders of magnitude was necessary for the other model parameters in order for the effect of sampling frequency to be negligible. It should also be noted that adaptation of $T_{\text{Gla}}$ and $T_S$ still proved impossible.

5.5.2.2 Introducing sampling noise

Introduction of sampling noise resulted in both model and parameter errors exhibiting oscillations within a certain band, which was expected. Furthermore, a ‘core’ was observed within that band of values, for all model parameter/measured output combinations explored. Therefore, for these simulations, the concept of ‘acceptable’ oscillations introduced in Section 4.3.2 was re-defined as oscillations with a core of average magnitude smaller than the initial parameter mismatch. The performance measures were defined as previously (Section 4.3.2), although they were calculated based on the average magnitude of the oscillations observed.

Model adaptation was successful for all model parameters manipulated, except $T_{\text{Gla}}$ and $T_S$. However, in most cases, $K_a$ had to be decreased by an order of magnitude relative to the optimal values, or the noise levels reduced down to 1%, in order to obtain performance measures similar to those observed without sampling noise (Tables 5.1 to 5.4). The parameter error obtained for adaptation of $R_{\text{Anti}}$ for a -10% initial mismatch, at a $K_a$ value of $6 \times 10^{-7}$ ml h$^{-1}$ cell$^{-1}$ (sub-optimal value) and a noise level of 1%, is presented in Figure 5.5, as an example. It can be seen that the band of values obtained for the parameter error exhibits a core of $\approx 8\%$; adaptation was thus successful at this noise level.
5.5.2.3 Introducing a hidden mismatch

Overall, adaptation was less sensitive to mismatches in $T_{GIA}$ than in $T_S$. Model adaptation proved impossible when introducing a hidden mismatch in $T_{GIA}$ or $T_S$ when using viable cell concentration as measured output. For all other measured outputs, model adaptation was successful when considering $\pm 4\%$ mismatches. It has been observed that $S_{Max}$ was the only model parameter which could be adapted when either $T_{GIA}$ or $T_S$ were doubled (+100% mismatch); no model parameter could be adapted when considering a -100% mismatch in $T_{GIA}$ or $T_S$. The results obtained when altering the cell flow were qualitatively similar to those obtained when altering $T_{GIA}$ or $T_S$ by $\pm 100\%$. These results were achieved at $K_a$ values smaller than the optimal values (Tables 5.1 to 5.4) for most cases considered; in general, a decrease of an order of magnitude was necessary.

The parameter error obtained for adaptation of $S_{Max}$ at the optimal $K_a$ value (Table 5.2) and for a -10% initial parameter mismatch, introducing a hidden mismatch of -4% in $T_{GIA}$, is presented in Figure 5.6; the error obtained without the hidden mismatch is included for comparison. It can be observed that model adaptation is possible both with and without a hidden mismatch. Both overshoot and ultimate error are greater in the presence of the hidden mismatch, but cross time is the same; no settling time can be determined.

5.5.2.4 Modifying several model adaptor action parameters

Simulations have also been conducted considering combinations of the action parameters addressed above. It has been observed that the effect of decreasing sampling frequency upon model adaptation was very small when also introducing sampling noise or a hidden mismatch; this matches the results obtained when only decreasing sampling frequency (Section 5.5.2.1). The parameter error obtained for adaptation of $R_{Anti}$ at a $K_a$ value of $6 \times 10^{-7}$ ml h$^{-1}$ cell$^{-1}$, for a -10% initial mismatch and a noise level of 1%, for sampling intervals of 6 min and 2 h, is presented in Figure 5.7, as an example. It can be seen that the error varies within the same ‘band’
for both sampling frequencies; furthermore, the values for the performances values are the same.

Introduction of both sampling noise and a hidden mismatch did not significantly affect the results obtained if the noise levels were no greater than 5% and the mismatch introduced was ±4% in $T_{G1a}$ or $T_S$. However, model adaptation became impossible for most of the combinations explored when also decreasing sampling frequency. The parameter error obtained for adaptation of $S_{max}$ at the optimal $K_a$ value and for a -10% initial parameter mismatch, introducing a hidden mismatch of -4% in $T_{G1a}$, for both 6 min and 2 h sampling intervals, is presented in Figure 5.8, as an example. It can be seen that model adaptation was possible for a sampling interval of 6 min, both when only considering the hidden mismatch and when also considering sampling noise. However, the differences in parameter error for different sampling intervals in the presence of noise were significant. According to the re-definition of acceptable oscillations stated in Section 5.5.2.2, model adaptation was considered to be impossible when considering sampling intervals of 2 h.

5.5.3 Discussion of results

The effect of decreasing sampling frequency upon model adaptation proved negligible for most manipulated parameters. This means that the changes in cell age distribution which occur between samplings were not significant even when sampling every 2 h. This is a vital finding in terms of the applicability of the model adaptor algorithm to an experimental situation as sampling every 2 h is feasible and allows for off-line measurements.

The introduction of sampling noise to process outputs affected the system to a greater extent than decreasing sampling frequency; for some of the combinations explored, adaptation was only possible for a noise level of 1%, which is an unrealistic value. It has been observed that both model and parameter errors exhibited a band of values and that a core could be identified within this band; this is due to the 'bell shape'
nature of a normal distribution. The noisy process output value has a high probability of being very similar to the non-noisy value (equivalent to the mean of the distribution), but also a low probability of being very different from the non-noisy value (the 'tails' of a normal distribution).

The Box-Müller method employed to introduce sampling noise yields normally distributed numbers between $-\infty$ and $+\infty$, exhibiting a variance of 1 (Section 5.5.1). A decrease in variance would correspond to a greater value of the density function for the mean value, but also to the density function exhibiting a narrower 'bell' base. Therefore, the noise added to the process output would always be very similar, which would be expected from sampling noise. It can thus be expected that, in a real situation, adaptation results would be better than those obtained in the simulations conducted. Therefore, model adaptation could be successful at the more realistic values of 5 and 10%.

Overall, introduction of a hidden mismatch affected model adaptation to a greater extent than decreasing sampling frequency or introducing sampling noise. This was expected as the hidden parameter mismatches considered are related to the model structure. It was observed that adaptation was less sensitive to mismatches in $T_{G1a}$ than in $T_S$; this is due to $T_{G1a}$ being half the value of $T_S$, as discussed in Section 4.5.2. The similarity between the results obtained by altering the duration of phases and those obtained by alteration of the flow of cells was also expected. The latter resulted in 50% of the cells leaving S going round the cell cycle faster than without the presence of the hidden parameter mismatch. Therefore, it corresponds to the duration of the G2 phase being altered by -100% for those cells, which explains the similarities observed. It was also observed that using viable cell concentration as measured output resulted in impossible model adaptation for all manipulated model parameters, regardless of the hidden parameter mismatch introduced. This is due to varying the durations of phases G1a, S or G2 affecting the distribution of cells in the cell cycle and, thus, resulting in greater model errors for viable cell concentration than for the other measured outputs considered.
Model adaptation was also investigated when considering combinations of the above action parameters. The results obtained for all the four combinations considered were similar to those obtained when the action parameters were addressed separately. The greatest effect upon model adaptation has been observed when considering all action parameters. This was particularly noticeable for sampling intervals of 2 h as the cell age distribution exhibited significant differences relative to that obtained for 6 min intervals when introducing both an error to the process outputs and an additional process/model mismatch.

Overall, the model parameter which coped better with all the features investigated was $R_{\text{Anti}}$ using antibody concentration as measured output. It may be noted that this was also the best combination in the preliminary testing of the model adaptor algorithm in repeated batch operation (Section 5.3).

### 5.6 Concluding Comments

The model adaptor algorithm has proven capable of adapting the model in repeated batch operation, when employing proportional-only action, except for manipulation of $T_{G1a}$ or $T_S$. Adaptation of $R_{\text{Glut}}$, $S_{\text{Max}}$, $R_{\text{Anti}}$ and $k$ were successful for some of the model parameter/measured output combinations explored. Overall, the best adaptation results were obtained by manipulation of $R_{\text{Anti}}$ using antibody concentration as measured output.

Qualitative similarities have been observed between the results obtained in repeated batch and those attained in batch operation. However, the values for the performance measures were, in general, better in repeated batch. This was due to the higher $K_a$ values used in repeated batch as a result of the changes in dynamics introduced whenever the culture was resuspended. This also led to the successful adaptation of $k$, which had not been possible in batch operation.
Further Testing of the Model Adaptor Algorithm

The model adaptor algorithm was further investigated in repeated batch operation by modifying some action parameters, either separately or in combination. It has been observed that decreasing sampling frequency to 2 h or introducing 1% sampling noise to the measured outputs had negligible effects on model adaptation. However, some limitations were observed when introducing a hidden parameter mismatch as the algorithm only coped with ±4% hidden mismatches in $T_{G_{1a}}$ or $T_S$. The greatest effect upon model adaptation was observed when simultaneously decreasing sampling frequency, introducing sampling noise and introducing a hidden parameter mismatch.

It is clear from the results presented in Chapters 4 and 5 that the model adaptor algorithm developed has great potential application for MBO control, both for batch and repeated batch operation. However, it should be pointed out that these results will only be useful if the process controller algorithm proves capable of controlling the process. The results obtained when testing this algorithm will be presented and discussed in the next chapter. Only repeated batch operation will be considered as results obtained in batch can be inferred from those obtained in repeated batch. Furthermore, repeated batch presents an additional challenge due to the potential occurrence of catastrophic failure and the profusion of process behaviours observed when changing the operational conditions (Section 3.5.3).
Chapter 6
Development and Testing of the Process Controller Algorithm

6.1 Introduction

Testing of the model adaptor algorithm has shown that the mm321 hybridoma cell line model considered can be adapted on-line, in both batch and repeated batch operation. Furthermore, it has been observed that batch results could be inferred from those obtained in repeated batch. It has been concluded that the model adaptor algorithm has great potential within the model-based observer (MBO) control strategy. However, the success of this control strategy is also dependent on the ability of the process controller to enhance process performance.

In this chapter, the methodology employed to develop and test the process controller algorithm will be detailed. Furthermore, the results obtained when testing the process controller algorithm in repeated batch will be presented and discussed. The algorithm was tested in order to investigate whether process performance could be optimised when applying model-based control and whether results would be better than employing conventional control.

6.2 Development of the Process Controller Algorithm

The purpose of feedback control is to drive process outputs towards set points in order to meet pre-specified objectives, mainly dictated by operational aims. The control objective adopted for this work was the enhancement of process performance and the
Development and Testing of the Process Controller Algorithm

Operational aim was the optimisation of antibody productivity, defined as the rate of antibody production per unit volume of bioreactor. As the bioreactor was simulated in repeated batch mode, optimisation of productivity was considered to be a compromise between maximising the overall productivity and minimising variations in productivity between consecutive resuspension cycles.

### 6.2.1 Control strategies

Process control may be achieved in different ways, depending on the control strategy adopted. A model-based control strategy and a conventional control strategy have been considered in this work in order to compare the capability of each strategy in optimising antibody productivity.

The conventional control strategy adopted consisted of manipulating a process parameter with respect to set point error, i.e., the difference between the set point value and the measured process output. The model-based control strategy adopted consisted of manipulating a process parameter with respect both to set point error and to process information inferred on-line by the model. It should be noted that manipulation with respect to set point error was conducted in the same way for both control strategies. The algorithm developed may thus be used for either control strategy.

A schematic of the model-based control strategy considered is presented in Figure 6.1; the grey lines refer to the elements from Figure 3.1 which were not considered at this stage. The model adaptor was considered to be switched off and, thus, exact model copies were employed in the process loop and in the model loop. Furthermore, no unknown disturbances were considered as the process was simulated. When employing conventional control, the information from the model used as input to the process controller was not considered.
6.2.2 Control action

The process controller manipulated a process parameter with respect to set point error as follows:

\[ z_p(t) = z_b(t) + c_c(t) \]  

(6.1)

where \( z_p(t) \) is the current manipulated process parameter value (units as appropriate) and \( z_b(t) \) is the bias value (units of the process parameter). The definition of the term \( c_c(t) \) (units of the process parameter) depends on the control action considered for the process controller. Proportional-only and proportional-integral control actions were considered; a derivative term was not considered due to the usual presence of noise associated with bioreactor operation. For proportional-only action, \( c_c(t) \) was defined as:

\[ c_c(t) = K_c [SP(t) - y_p(t)] \]  

(6.2)

and for proportional-integral action:

\[ c_c(t) = K_c \left\{ [SP(t) - y_p(t)] + \frac{1}{\tau_c} \int_{0}^{1} [SP(t) - y_p(t)] \, dt \right\} \]  

(6.3)

where \( K_c \) is the proportional gain of the process controller (units defined by the units of the process parameter and of the outputs); \( \tau_c \) is the integral action time of the process controller (units defined by the units of the process parameter and of the outputs); \( y_p(t) \) is the current process output (units as appropriate) and \( SP(t) \) is the set point value (units of the process output). \( K_c \) and \( \tau_c \) will be referred to as the process controller parameters.

In principle, any operational condition (e.g.: pH, temperature, harvest fraction,
Development and Testing of the Process Controller Algorithm

dilution cycle time) could be chosen to be a manipulated process parameter. It has been demonstrated (Section 3.5.3) that repeated batch operation is very sensitive to changes in harvest fraction (HF) and dilution cycle time (DCT). The latter has been chosen as it is easier to alter in operational terms than HF. It should be noted that the algorithm only allowed integer DCT values as in a real situation it would be practical to resuspend on an hourly basis.

The bias in Equation 6.1 was the DCT value at the beginning of each resuspension cycle. It changes from one cycle to the next in order to ensure 'bump-free' operation, similar to the bias defined for the model parameter value, for repeated batch operation (Section 4.2.1).

The set point was an antibody concentration which changed with time in order to meet an overall 'desired' antibody production rate of 0.5 µg ml⁻¹ h⁻¹. This is referred to as a servo control problem (Stephanopoulos, 1985; Seborg et al., 1989) as the process output tracks a changing set point (Section 2.4.1); the set point was thus indicated as time dependent in Equations 6.2 and 6.3. The process output chosen was the antibody concentration in the medium, so that it could be compared to the set point. The set point error, \( e_{SP}(t) \), was thus given by:

\[
e_{SP}(t) = SP(t) - y_p(t)
\]  

(6.4)

where \( SP(t) \) is the set point (mg ml⁻¹) and \( y_p(t) \) is the antibody concentration obtained from the process (mg ml⁻¹).

The additional information provided on-line by the model to manipulate the process parameter was the cell age distribution. However, its use for controlling the process is not straightforward due to the complexity of this information. It should be noted that analysis of complex data is beyond the context of this work. The use of cell age distribution in terms of ratios between the fraction of cells in different phases was alternatively considered; either information is specific to the case study considered.
6.3 Testing of the Process Controller Algorithm

The process controller algorithm was tested in repeated batch over 100 resuspension cycles, for an initial viable cell concentration of $0.09 \times 10^{+06}$ cell ml$^{-1}$ and an initial glutamine concentration of 0.1 mg ml$^{-1}$, at a HF value of 70% and an initial DCT value of 50 h; the inoculum state was that described in Section 3.5.1. The process was simulated and controlled on-line, using a sampling frequency of 6 min. Further investigation of the process controller algorithm included introducing or changing: sampling frequency, sampling noise, hidden mismatch, set point and delays prior to resuspension.

6.3.1 Cell ratio information

As stated above, the use of cell ratios for manipulating DCT depends on the case study considered, i.e., on the initial and operational conditions selected. The viable cell concentration obtained for the process with no control action and the ratio between the fraction of cells in G1b and the fraction of cells in D are presented in Figure 6.2, for the first 25 resuspension cycles. The viable cell concentration had already been presented in Figure 3.9 (Section 3.5.3), although more values per cycle are shown here. It can be observed that dramatic variations in cell growth in consecutive cycles are immediately preceded by a peak in the ratio value. A high value of the ratio means that there are many cells in G1b. Therefore, it can be concluded that the large decreases in viable cell concentration result from too many cells being in G1b, exhausting glutamine. This results in cells accumulating in G1b, subsequently dying; thus, the G1b/D cell ratio decreases significantly. Therefore, this ratio may be regarded as a predictor of catastrophic failure in the following cycle.

It was proposed from this analysis that this ratio could be used as additional information to control the process. Therefore, the process controller manipulates DCT with respect to the set point error, but also to this ratio. The former action occurs at each sampling (Equations 6.1 to 6.3), whereas the latter alters DCT such that the
culture is resuspended when and if certain ratio values are reached. A band of permissible ratio values was pre-defined as the interval [0,6] based on the preliminary results illustrated in Figure 6.2. Culture resuspension occurred whenever the ratio exhibited a peak, for ratio values exceeding the upper limit of the pre-defined band. However, it should be noted that resuspension was not necessarily immediate due to the restriction of integer values imposed on DCT, as stated in Section 6.2.2.

6.3.2 Analysis of results

The process response to control action was investigated by analysing the variation with time of antibody concentration, viable cell concentration, viability and set point error. Antibody and viable cell concentrations were analysed in terms of the initial and final values in each cycle; viability and set point error, in terms of their final value. These analyses were adopted for practical reasons of presentation of the data obtained, although all these values were available at each sampling interval.

Antibody concentration data was also used to determine the productivity attained. The overall productivity was calculated as the cumulative concentration of harvested antibody divided by the total duration of the resuspension cycles, referred to as the total production time.

A common statistical analysis of the data obtained was conducted for both control strategies. The descriptive statistics considered for the distribution of values of antibody concentration, viable cell concentration, viability and set point error obtained were the mean, standard deviation and skewness. The mean is the average value obtained; the standard deviation is a measure of how widely values are dispersed from the mean; and skewness characterises the degree of asymmetry of the distribution of values around the mean. Positive skewness indicates higher probability of occurrence of values lower than the mean; negative skewness indicates higher probability for values greater than the mean; and a skewness value of zero corresponds to a normal
distribution of values (Spiegel, 1976). The values obtained for standard deviation and skewness may be regarded as broad measures of data variability.

It is desirable to obtain small values for standard deviation and skewness and large values for overall productivity and the mean values for all output variables. Negative set point errors, of large absolute value, are desirable as they correspond to greater antibody production relative to the value indicated by the set point. It should be noted that the process controller parameters may result in desirable values for the descriptive statistics, whilst yielding low productivity and/or high degree of variation in productivity between consecutive cycles.

The criteria adopted for successful process control were the maximisation of overall productivity whilst minimising variations in productivity between consecutive cycles. The process controller parameters were optimised in order to achieve these criteria; they were designated as optimal $K_c$ and optimal $\tau_c$. However, the optimisation was only conducted in broad terms, similarly to the determination of the optimal model adaptor parameters (Section 4.3.2).

### 6.4 Results

The viable cell concentration, viability and antibody concentration obtained with no control action, at the initial and operational conditions considered, are presented in Figure 6.3. It can be observed that these conditions resulted in frequent occurrence of catastrophic failure. These HF/DCT values were selected in order to investigate whether the process controller algorithm could eliminate or considerably reduce the occurrence of catastrophic failure and, thus, optimise the antibody productivity.

As explained in Section 6.2.1, both conventional and model-based control strategies have been considered for testing the algorithm; the results obtained will be presented in Sections 6.4.1 and 6.4.2, respectively. The process was simulated and controlled
Development and Testing of the Process Controller Algorithm

on-line, considering sampling intervals of 6 min, for both control strategies. When applying model-based control, the culture was resuspended when the G1b/D cell ratio exhibited a peak for values greater than 6 or within an hour of that occurring due to the restriction of integer values imposed on DCT (Section 6.2.2).

6.4.1 Conventional control strategy

Overall, the process controller algorithm proved capable of enhancing process performance when applying conventional control, for proportional-only action. $K_c$ was varied between 5 and 100 h ml mg$^{-1}$. Values lower than, or equal to, 20 h ml mg$^{-1}$ had no effect on the process; unrecoverable catastrophic failure was observed for a $K_c$ value of 100 h ml mg$^{-1}$. Recoverable catastrophic failure was exhibited for $K_c$ values between 20 and 100 h ml mg$^{-1}$.

The overall productivity, total production time and descriptive statistics defined for all output variables and for set point error are presented in Table 6.1 (Page 92), for several $K_c$ values. As stated before, no effect on the process is observed at $K_c$ values lower than, or equal to, 20 h ml mg$^{-1}$; unrecoverable catastrophic failure is observed at 100 h ml mg$^{-1}$. For all other $K_c$ values, an increase in $K_c$ resulted in an increase in productivity, except when $K_c$ was increased from 20 to 25 h ml mg$^{-1}$ and from 50 to 60 h ml mg$^{-1}$; productivity was greater than that attained with no control action for all $K_c$ values except 25 h ml mg$^{-1}$. An increase in $K_c$ also resulted in a decrease in production time, except when $K_c$ was increased from 50 to 60 h ml mg$^{-1}$. It can be seen in Table 6.1 that the highest productivity was obtained at a $K_c$ value of 50 h ml mg$^{-1}$, corresponding to a 43% enhancement relative to the productivity attained with no control action; total production time decreased by 1%.

The mean for all measured outputs and for set point error is presented in Figure 6.4. It can be seen that the mean values for antibody and viable cell concentrations were always greater than those observed under no control action, except at 25 h ml mg$^{-1}$; the mean for viability remained slightly higher than the value obtained with no control
action. It can also be seen that, for $K_c$ values between 20 and 100 h ml mg$^{-1}$, an increase in $K_c$ resulted in an increase in mean for antibody and viable cell concentrations and in a decrease in mean for set point error, except when $K_c$ was increased from 20 to 25 h ml mg$^{-1}$ and from 50 to 60 h ml mg$^{-1}$. An increase in $K_c$ had no significant effect on the mean value for viability.

It can be seen in Table 6.1 that, overall, an increase in mean for both antibody and viable cell concentrations corresponds to a decrease in standard deviation and skewness. An increase in mean for set point error corresponds to an increase in standard deviation. No clear relationships can be identified between variations in mean and skewness for set point error or between the descriptive statistics defined for viability.

The variation in viable cell concentration, viability, antibody concentration, set point error and DCT obtained at 95 and at 50 h ml mg$^{-1}$ are presented in Figures 6.5 and 6.6, respectively; these $K_c$ values resulted in the highest productivity values obtained (Table 6.1). It can be seen that catastrophic failure occurred at both $K_c$ values. However, a significant reduction can be observed relative to the process outputs obtained with no control action (Figure 6.3). This is especially noticeable at 50 h ml mg$^{-1}$ as both viable cell concentration and viability are significantly more stable; this behaviour is reflected in both antibody concentration and set point error. Furthermore, larger variations in DCT relative to its ‘original’ value, i.e., 50 h are observed at 95 h ml mg$^{-1}$. However, DCT was manipulated by small amounts for both $K_c$ values, which resulted in small variation in total production time (Table 6.1).

The process controller algorithm was also tested employing proportional-integral action. Application of this control action was conducted as described for the model adaptor (Section 4.4.2.1); a wide range of $\tau_c$ values was used. In general, introduction of an integral term resulted in unrecoverable catastrophic failure of the culture and in a great decrease in overall productivity relative to that obtained with no control action. It has been observed that $K_c$ and $\tau_c$ had opposite effects on process response.
Furthermore, for the same $\tau_c$, an increase in $K_c$ corresponded to unrecoverable catastrophic failure occurring earlier in the simulation.

### 6.4.2 Model-based control strategy

Overall, the process controller algorithm proved capable of enhancing process performance when applying model-based control, for proportional-only action. $K_c$ was varied between 5 and 100 h ml mg$^{-1}$. The process behaviour exhibited at all $K_c$ values was different from that observed with no control action. Values lower than, or equal to, 30 h ml mg$^{-1}$ had the same effect upon the process outputs. Washout was observed for $K_c$ values greater than, or equal to, 60 h ml mg$^{-1}$.

The overall productivity, total production time and descriptive statistics defined for all output variables and for set point error are presented in Table 6.2 (Page 93), for several $K_c$ values. It can be seen that productivity was greater than that attained with no control action for $K_c$ values lower than 60 h ml mg$^{-1}$. As stated above, $K_c$ values lower than, or equal to, 30 h ml mg$^{-1}$ had the same effect upon output variables and set point error. For greater $K_c$ values, an increase in $K_c$ resulted in a decrease in productivity, except when $K_c$ was increased from 45 to 50 h ml mg$^{-1}$ and from 90 to 100 h ml mg$^{-1}$. An increase in $K_c$ also resulted in an increase in production time, except when $K_c$ was increased from 30 to 42 h ml mg$^{-1}$ and from 90 to 100 h ml mg$^{-1}$. It can be seen in Table 6.2 that the highest productivity was obtained for $K_c$ values lower than, or equal to, 30 h ml mg$^{-1}$. This productivity corresponds to a 90% increase relative to that attained with no control action; total production time decreased by 21%.

The mean for all measured outputs and for set point error is presented in Figure 6.7. It can be observed that $K_c$ values lower than 60 h ml mg$^{-1}$ resulted in mean values for antibody concentration, viable cell concentration and viability greater than those observed with no control action. It can also be seen that, for $K_c$ values greater than 30 h ml mg$^{-1}$, an increase in $K_c$ resulted in a decrease in mean for antibody and viable
cell concentrations and in an increase in mean for set point error, except when \( K_c \) was increased from 45 to 50 h ml mg\(^{-1}\). An increase in \( K_c \) resulted in a decrease in mean for viability, except when \( K_c \) was increased from 40 to 42 h ml mg\(^{-1}\) and from 60 to 80 h ml mg\(^{-1}\).

It can be seen in Table 6.2 that an increase in mean for antibody and viable cell concentrations corresponds to a decrease in standard deviation, except when \( K_c \) was increased from 30 to 42 h ml mg\(^{-1}\) and from 90 to 100 h ml mg\(^{-1}\); it also corresponds to a decrease in skewness, except when \( K_c \) was increased from 30 to 40 h ml mg\(^{-1}\) and from 90 to 100 h ml mg\(^{-1}\). An increase in mean for viability corresponds to a decrease in standard deviation, except when \( K_c \) was increased from 30 to 42 h ml mg\(^{-1}\) and from 45 to 50 h ml mg\(^{-1}\); to a decrease in skewness, except when \( K_c \) was increased from 40 to 50 h ml mg\(^{-1}\). It can also be seen that an increase in mean for set point error corresponds to an increase in standard deviation for \( K_c \) values lower than 60 h ml mg\(^{-1}\); to a decrease for greater \( K_c \) values. It also corresponds to an increase in skewness, except when \( K_c \) was increased from 40 to 42 h ml mg\(^{-1}\), from 43 to 45 h ml mg\(^{-1}\) and from 70 to 80 h ml mg\(^{-1}\).

The variation in viable cell concentration, viability, antibody concentration, set point error and DCT obtained at 45 and 30 h ml mg\(^{-1}\) are presented in Figures 6.8 and 6.9, respectively. It can be seen in Figure 6.8a that a \( K_c \) value of 45 h ml mg\(^{-1}\) resulted in a considerable reduction in the occurrence of catastrophic failure relative to the results obtained with no control action (Figure 6.3). However, at a \( K_c \) value of 30 h ml mg\(^{-1}\), no catastrophic failure occurred; in fact, it can be observed that cell growth within a cycle remains approximately constant throughout the simulation. This behaviour is reflected in the variation exhibited by antibody concentration (Figures 6.8b and 6.9b) and set point error (Figures 6.8c and 6.9c). Furthermore, larger and more frequent variations in DCT relative to its ‘original’ value, i.e., 50 h are observed at 45 h ml mg\(^{-1}\). This resulted in a 12% decrease in total production time relative to that observed at 30 h ml mg\(^{-1}\).

Testing of the process controller algorithm was also conducted considering
<table>
<thead>
<tr>
<th>Overall productivity (µg ml⁻¹ h⁻¹)</th>
<th>Kₚ (h ml mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total production time (h)</td>
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</tr>
<tr>
<td>No control action</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
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<td>0.630</td>
<td>0.630</td>
</tr>
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<td>5000</td>
<td>5000</td>
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**DESCRIPTIVE STATISTICS**

<table>
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<tr>
<th>Antibody concentration (µg ml⁻¹)</th>
<th>mean (µg ml⁻¹)</th>
<th>standard deviation (%)</th>
<th>skewness</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean (µg ml⁻¹)</td>
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<td>81</td>
<td>0.94</td>
</tr>
<tr>
<td>standard deviation (%)</td>
<td>29.2</td>
<td>81</td>
<td>0.94</td>
</tr>
<tr>
<td>skewness</td>
<td>29.2</td>
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<td>0.94</td>
</tr>
<tr>
<td>mean (µg ml⁻¹)</td>
<td>29.2</td>
<td>81</td>
<td>0.94</td>
</tr>
<tr>
<td>standard deviation (%)</td>
<td>29.2</td>
<td>81</td>
<td>0.94</td>
</tr>
<tr>
<td>skewness</td>
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<td>0.94</td>
</tr>
<tr>
<td>Viability (%)</td>
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<td>skewness</td>
</tr>
<tr>
<td>mean (%)</td>
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<td>-0.95</td>
</tr>
<tr>
<td>standard deviation (%)</td>
<td>0.119</td>
<td>83</td>
<td>-0.95</td>
</tr>
<tr>
<td>skewness</td>
<td>0.119</td>
<td>83</td>
<td>-0.95</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>mean (%)</td>
<td>standard deviation (%)</td>
<td>skewness</td>
</tr>
<tr>
<td>mean (%)</td>
<td>0.119</td>
<td>83</td>
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</tr>
<tr>
<td>standard deviation (%)</td>
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<td>83</td>
<td>-0.95</td>
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<tr>
<td>skewness</td>
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<td>83</td>
<td>-0.95</td>
</tr>
<tr>
<td>Set point error (µg ml⁻¹)</td>
<td>mean (µg ml⁻¹)</td>
<td>standard deviation (%)</td>
<td>skewness</td>
</tr>
<tr>
<td>mean (µg ml⁻¹)</td>
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<tr>
<td>standard deviation (%)</td>
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<td>skewness</td>
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<td>307</td>
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Table 6.1 - Productivity, production time and descriptive statistics determined with no control action and when applying conventional control, for proportional-only action.
### Development and Testing of the Process Controller Algorithm

<table>
<thead>
<tr>
<th>Overall productivity (µg ml⁻¹ h⁻¹)</th>
<th>No control action</th>
<th>Kₙ (h ml mg⁻¹)</th>
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<tr>
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<td>1.20</td>
</tr>
<tr>
<td>Total production time (h)</td>
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<td>3935</td>
</tr>
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<table>
<thead>
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<th>DESCRIBITIVE STATISTICS</th>
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</thead>
<tbody>
<tr>
<td><strong>Antibody concentration</strong> (µg ml⁻¹)</td>
<td>mean (µg ml⁻¹)</td>
<td>29.2</td>
</tr>
<tr>
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<td>standard deviation (%)</td>
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</tr>
<tr>
<td></td>
<td>skewness</td>
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</tr>
<tr>
<td><strong>Viability</strong> (%)</td>
<td>mean (%)</td>
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</tr>
<tr>
<td></td>
<td>standard deviation (%)</td>
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</tr>
<tr>
<td></td>
<td>skewness</td>
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</tr>
<tr>
<td><strong>Set point error</strong> (µg ml⁻¹)</td>
<td>mean (µg ml⁻¹)</td>
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</tr>
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<td>standard deviation (%)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>skewness</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

**Table 6.2** - Productivity, production time and descriptive statistics determined with no control action and when applying model-based control, for proportional-only action.
proportional-integral action; \( \tau_c \) was varied within a wide range of values. Introduction of an integral term resulted in the washout of the bioreactor contents, regardless of the values used for the process controller parameters. Furthermore, the productivity attained was much smaller than that obtained with no control action. It has been observed that an increase in \( \tau_c \) had the opposite effect of an increase in \( K_c \). However, for the same \( \tau_c \), an increase in \( K_c \) corresponded to washout occurring earlier in the simulation.

6.5 Discussion of Results

It is clear from the above results that process performance could be enhanced applying either control strategy. This was achieved by manipulation of DCT in order to reach a value so that catastrophic failure would not occur or would be less frequent. The aim of this section is to analyse and discuss all results obtained, comparing the results obtained employing each control strategy.

Application of conventional control, employing proportional-only action, did not eliminate the occurrence of catastrophic failure observed with no control action, although a significant reduction was observed at some \( K_c \) values. The results obtained for \( K_c \) values lower than, or equal to, 20 h ml mg\(^{-1}\) match those observed with no control action due to these \( K_c \) values being too small to manipulate DCT. For \( K_c \) values between 20 and 100 h ml mg\(^{-1}\), less catastrophic failure occurred relative to that observed with no control action due to less frequent glutamine exhaustion. High fractions of cells in the G1b phase result in glutamine exhaustion, which is the limiting nutrient (Section 3.4.2), but also in high antibody production. Over-production of antibody relative to the set point value corresponds to a negative set point error; those \( K_c \) values resulted in large decreases in DCT, which corresponded to addition of fresh medium occurring sooner than with no control action. Therefore, it can be concluded that catastrophic failure occurred less frequently due to
At a $K_c$ value of 100 h ml mg$^{-1}$, DCT was changed by greater amounts for the same set point error than at lower $K_c$ values. Whenever under-production of antibody occurred, this $K_c$ value dictated large values for DCT; thus, the cell population had more time to produce antibody so as to match the set point. However, these DCT values were too large and led to exhaustion of glutamine. Subsequently, the whole cell population irreversibly entered the D phase and died, resulting in unrecoverable catastrophic failure.

Overall, application of model-based control, employing proportional-only action, resulted in better process performance than applying conventional control. In principle, this was due to DCT being manipulated with respect to both the set point error and the G1b/D cell ratio; in other words, with respect to both antibody production and glutamine availability.

High G1b/D ratio values indicate there is a large number of cells in G1b; this is due to accumulation of cells in G1b as a result of glutamine exhaustion. Resuspending the culture when the ratio exhibited a peak, for values greater than 6, resulted in less cells dying due to more frequent addition of fresh medium. It has been observed that the set point error had no effect on DCT and thus process performance for $K_c$ values lower than, or equal to, 30 h ml mg$^{-1}$. Greater $K_c$ values resulted in an effect upon DCT and a decrease in productivity was observed. It can thus be concluded that the set point error has a detrimental effect upon process performance.

The effect of set point error on DCT was particularly noticeable for $K_c$ values greater than 50 h ml mg$^{-1}$. These $K_c$ values led to washout of the bioreactor contents, resulting in a sharp decrease in productivity. This was due to those $K_c$ values dictating variations in DCT which led to small cell populations of high viability. Consequently, the G1b/D ratio was high, which dictated early resuspension and, thus, little cell growth, leading to washout. It can, thus, be concluded from the results obtained that
there is no need to determine the set point error when the GIb/D cell ratio information is available.

An extract of the variation in the GIb/D cell ratio obtained with no control action and at Kc values of 45 and 30 h ml mg⁻¹ is presented in Figure 6.10. It can be seen that catastrophic failure occurs at ≈100 h with no control action, but not when applying model-based control, for either Kc value considered. The elimination of catastrophic failure was due to the manipulation of DCT with respect to the ratio value, for both Kc values. As discussed above, DCT can also be manipulated with respect to set point error for a Kc value of 45 h ml mg⁻¹. It can be seen in Figure 6.10b that, for this Kc value, set point error subsequently dictated large DCT values to allow larger antibody production times in order to meet the set point value. At this point, the ratio value was within its pre-defined band and, thus, did not dictate changes in the DCT value. Manipulation of DCT with respect to set point error resulted in a high fraction of cells in GIb, leading to glutamine exhaustion and subsequent catastrophic failure (Figure 6.8). Therefore, the effect of set point error upon process performance was not always beneficial at this Kc value. In fact, it can be concluded that better results were obtained at 30 h ml mg⁻¹, when set point error had no effect on DCT and, thus, process performance.

It is clear from Table 6.1 that the highest overall productivity when applying conventional control was obtained at 50 h ml mg⁻¹. Furthermore, the variability measures for antibody concentration indicate that this Kc value also resulted in the lowest degree of variation in productivity between consecutive cycles. Therefore, it can be concluded that this Kc value resulted in the best optimisation of process performance. According to the criteria adopted for successful process control, stated in Section 6.3.2, this value was considered to be the optimal Kc value. When applying model-based control, Kc values between 5 and 30 h ml mg⁻¹ resulted in maximisation of productivity whilst minimising variability. It may be noted that the same results would be obtained for Kc values between 0 and 5 h ml mg⁻¹, for the reasons explained before. However, as discussed above, set point error has no effect on DCT at these Kc
values; thus, no optimal \( K_c \) value was defined. It was concluded that the best results were obtained considering uniquely the \( G_{lb}/D \) ratio information to manipulate DCT.

Overall, the introduction of an integral term to the process controller action was not beneficial when applying either of the control strategies. It led to unrecoverable catastrophic failure when conventional control was employed and to washout, for model-based control. These results were due to accumulation and amplification of errors. Proportional-integral action involves calculation of the integral of the set point error, which corresponds to determining a cumulative term for the error. This accumulation dictates greater variations in DCT than those obtained by employing proportional-only action, even when \( \tau_c \) is large (Equations 6.2 and 6.3). It has also been observed that an increase in \( \tau_c \) has the same effect upon the process as a decrease in \( K_c \), for both control strategies; this is in agreement with conventional control theory (Stephanopoulos, 1985; Seborg et al., 1989).

In conclusion, model-based control proved better than conventional control in ‘finding’ DCT values which result in avoiding the occurrence of catastrophic failure. The highest overall productivity attained when applying model-based control was twice the value obtained for conventional control and less variation in productivity between consecutive cycles was observed. It has also been concluded that the process should be controlled uniquely based on the information inferred on-line by the model, without determining the set point error.

### 6.6 Further Investigation

Further investigation of the process controller algorithm addressed three areas. First, process controller action parameters were investigated: sampling frequency, sampling noise and hidden mismatches; the latter was only investigated when employing model-based control. This testing was similar to that conducted for the model adaptor algorithm: the methodology employed was the same and was described in
Section 5.5.1. The aim of this investigation was to assess the capability of the process controller algorithm to deal with more realistic features. It should be noted that the results presented in Section 6.4 were obtained considering a sampling interval of 6 min, no sampling noise and no hidden mismatches. Secondly, process controller algorithm parameters were addressed: set point, the pre-defined band for the G1b/D cell ratio and delays prior to resuspension; conventional control was only employed to analyse the former. The aim was to investigate whether better results than those presented in Section 6.4 could be achieved. Finally, the algorithm was also tested for other case studies, i.e., using different HF/DCT values. The operational conditions employed led to unrecoverable catastrophic failure or washout with no control action. The aim was to investigate whether the algorithm was capable of dealing with these process behaviours and enhancing process performance.

DCT was manipulated employing proportional-only action. When employing conventional control, the optimal $K_c$ value determined previously (Section 6.5) was used, except for different HF/DCT values. When employing model-based control, the set point error was not considered to manipulate DCT, based on the discussion of the previous results (Section 6.5). Sampling frequency and sampling noise affect the value of the set point error, but not that of the G1b/D cell ratio. Therefore, the effect of these action parameters was not investigated when employing model-based control.

The algorithm was also tested using $K_c$ values between 5 and 100 h ml mg$^{-1}$ to investigate whether the optimal $K_c$ value would change or if the set point error would not have a detrimental effect on process performance, when considering the above features. It has been observed that the previous conclusions remained valid. Unless otherwise stated, it should be assumed that the results presented below were obtained at the optimal $K_c$ value, when applying conventional control and not determining set point error, when applying model-based control. An overview of the results obtained will be presented in Sections 6.6.1 to 6.6.3, according to the parameters investigated, followed by a general discussion in Section 6.6.4.
6.6.1 Results obtained when modifying process controller action parameters

The main results presented in this section are tabulated in Tables 6.3 and 6.4 (Pages 100 and 101) for, respectively, conventional and model-based control.

6.6.1.1 Decreasing sampling frequency

Antibody concentration was considered to be sampled at intervals of 1, 2 and 4 h, when applying conventional control; it may be noted that the latter value was not considered when investigating the model adaptor algorithm (Section 5.5.1). It can be seen in Table 6.3 that a decrease in sampling frequency resulted in a significant decrease in productivity; the means for antibody and viable cell concentrations were lower and the variability measures greater than the values obtained using a sampling interval of 6 min.

6.6.1.2 Introducing sampling noise

Noise levels of 1, 5 and 10% were introduced to antibody concentration, when applying conventional control, similarly to when testing the model adaptor algorithm (Section 5.5.1); sampling intervals between 6 min and 4 h were considered. The values presented in Table 6.3 refer only to the data obtained for a sampling frequency of 6 min as it has been observed that the effect of decreasing sampling frequency was similar to that presented in the previous section. It can be seen that the effect of introducing noise on process performance was considerable at any noise level.

6.6.1.3 Introducing a hidden mismatch

Hidden mismatches were introduced by modifying the structure of the model and not attempting to correct the mismatch thus introduced. The three mismatches introduced
### Table 6.3 - Further investigation of the process controller algorithm: results obtained when applying conventional control. (The base case refers to 6 min sampling interval and no noise.)

<table>
<thead>
<tr>
<th></th>
<th>Base case</th>
<th>Sampling interval (h)</th>
<th>Sampling noise (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Overall productivity (µg ml⁻¹ h⁻¹)</td>
<td>0.904</td>
<td>0.777</td>
<td>0.728</td>
</tr>
<tr>
<td>Total production time (h)</td>
<td>4945</td>
<td>4987</td>
<td>4992</td>
</tr>
<tr>
<td>DESCRIBITIVE STATISTICS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody concentration (µg ml⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (µg ml⁻¹)</td>
<td>41.4</td>
<td>35.9</td>
<td>33.6</td>
</tr>
<tr>
<td>standard deviation (%)</td>
<td>61</td>
<td>66</td>
<td>69</td>
</tr>
<tr>
<td>skewness</td>
<td>0.31</td>
<td>0.58</td>
<td>0.69</td>
</tr>
<tr>
<td>Viable cell concentration (cell ml⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (10⁶ cell ml⁻¹)</td>
<td>0.176</td>
<td>0.149</td>
<td>0.139</td>
</tr>
<tr>
<td>standard deviation (%)</td>
<td>61</td>
<td>68</td>
<td>72</td>
</tr>
<tr>
<td>skewness</td>
<td>0.33</td>
<td>0.54</td>
<td>0.64</td>
</tr>
</tbody>
</table>
Table 6.4 - Further investigation of the process controller algorithm: results obtained when applying model-based control. (The base case refers to no hidden mismatch.)

<table>
<thead>
<tr>
<th>DESCRiptive Statistics</th>
<th>antibody concentration (µg ml⁻¹)</th>
<th>Viable cell concentration (cell ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall productivity (µg ml⁻¹ h⁻¹)</td>
<td>1.20</td>
<td>0.218</td>
</tr>
<tr>
<td>Total production time (h)</td>
<td>3935</td>
<td>0.218</td>
</tr>
<tr>
<td>Base case</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hidden mismatch in</td>
<td>T_{G1a} (%)</td>
<td>T_{S} (%)</td>
</tr>
<tr>
<td></td>
<td>-4</td>
<td>+4</td>
</tr>
<tr>
<td>Overall productivity (µg ml⁻¹ h⁻¹)</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>Total production time (h)</td>
<td>3935</td>
<td>3935</td>
</tr>
<tr>
<td>Antibody concentration (µg ml⁻¹)</td>
<td>mean (µg ml⁻¹)</td>
<td>43.7</td>
</tr>
<tr>
<td>Antibody concentration (µg ml⁻¹)</td>
<td>standard deviation (%)</td>
<td>55</td>
</tr>
<tr>
<td>Antibody concentration (µg ml⁻¹)</td>
<td>skewness</td>
<td>0</td>
</tr>
<tr>
<td>Viable cell concentration (cell ml⁻¹)</td>
<td>mean (10⁶ cell ml⁻¹)</td>
<td>0.218</td>
</tr>
<tr>
<td>Viable cell concentration (cell ml⁻¹)</td>
<td>standard deviation (%)</td>
<td>54</td>
</tr>
<tr>
<td>Viable cell concentration (cell ml⁻¹)</td>
<td>skewness</td>
<td>0</td>
</tr>
</tbody>
</table>
were the same as those considered when further testing the model adaptor algorithm (Section 5.5.1).

It can be seen in Table 6.4 that mismatches of ±4% in either $T_{G1a}$ or $T_S$ did not affect process performance. However, performance decreased considerably when ±100% mismatches were considered, i.e., when phases were eliminated from the cell cycle or had their duration doubled. It can be observed from Table 6.4 that process performance was less sensitive to changes in $T_{G1a}$ than in $T_S$; this had also been observed for the model adaptor algorithm (Section 5.5.2.3). Process control proved poor when altering the flow of cells leaving the S phase as it resulted in washout of the bioreactor contents; this corresponded to a decrease of 66% in productivity.

6.6.2 Results obtained when modifying process controller algorithm parameters

6.6.2.1 Changing the set point

All the results previously presented in this chapter were obtained considering an overall 'desired' antibody production rate of 0.5 $\mu$g ml$^{-1}$ h$^{-1}$ to define the changing set point value, as stated in Section 6.2.2. The algorithm was further investigated by considering different production rates: 0.01, 0.1 and 1 $\mu$g ml$^{-1}$ h$^{-1}$; both control strategies were considered.

When applying conventional control, changing the production rate resulted in greater occurrence of catastrophic failure and led to unrecoverable catastrophic failure. The overall productivity, total production time and descriptive statistics obtained for antibody and viable cell concentrations are presented in Table 6.5. It can be observed that the 'original' production rate, i.e., 0.5 $\mu$g ml$^{-1}$ h$^{-1}$ resulted in the highest productivity. However, the overall productivity exceeded the rate value in all cases, except for 1 $\mu$g ml$^{-1}$ h$^{-1}$. It can also be seen in Table 6.5 that an increase in
<table>
<thead>
<tr>
<th>Overall productivity (µg ml⁻¹ h⁻¹)</th>
<th>Set point</th>
<th>Overall production rate (µg ml⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>0.458</td>
<td>0.296</td>
<td>0.904</td>
</tr>
<tr>
<td>Total production time (h)</td>
<td>4924</td>
<td>4959</td>
</tr>
<tr>
<td>Antibody concentration (µg ml⁻¹)</td>
<td>mean</td>
<td>20.9</td>
</tr>
<tr>
<td>standard deviation (%)</td>
<td>116</td>
<td>168</td>
</tr>
<tr>
<td>skewness</td>
<td>1.52</td>
<td>1.90</td>
</tr>
<tr>
<td>Viable cell concentration (cell ml⁻¹)</td>
<td>mean (10⁶ cell ml⁻¹)</td>
<td>0.096</td>
</tr>
<tr>
<td>standard deviation (%)</td>
<td>127</td>
<td>174</td>
</tr>
<tr>
<td>skewness</td>
<td>1.75</td>
<td>1.95</td>
</tr>
</tbody>
</table>

**Table 6.5** - Further investigation of the process controller algorithm: results obtained when applying conventional control, for different set points. (The shaded values refer to the 'original' set point.)

<table>
<thead>
<tr>
<th>Overall productivity (µg ml⁻¹ h⁻¹)</th>
<th>Set point</th>
<th>Overall production rate (µg ml⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>0.268</td>
<td>0.523</td>
<td>1.20</td>
</tr>
<tr>
<td>Total production time (h)</td>
<td>2890</td>
<td>3099</td>
</tr>
<tr>
<td>Antibody concentration (µg ml⁻¹)</td>
<td>mean</td>
<td>7.19</td>
</tr>
<tr>
<td>standard deviation (%)</td>
<td>253</td>
<td>144</td>
</tr>
<tr>
<td>skewness</td>
<td>2.70</td>
<td>1.49</td>
</tr>
<tr>
<td>Viable cell concentration (cell ml⁻¹)</td>
<td>mean (10⁶ cell ml⁻¹)</td>
<td>0.037</td>
</tr>
<tr>
<td>standard deviation (%)</td>
<td>254</td>
<td>143</td>
</tr>
<tr>
<td>skewness</td>
<td>2.74</td>
<td>1.48</td>
</tr>
</tbody>
</table>

**Table 6.6** - Further investigation of the process controller algorithm: results obtained when applying model-based control, for different set points. (The shaded values refer to the 'original' set point.)
Development and Testing of the Process Controller Algorithm

productivity was accompanied by an increase in mean for antibody and viable cell concentrations and a decrease in standard error and skewness.

Application of model-based control resulted in washout for production rate values lower than 0.5 μg ml⁻¹ h⁻¹; an increase in production rate resulted in washout occurring later in the simulation. It has also been observed that considering production rates of 0.5 and 1 μg ml⁻¹ h⁻¹ led to the same process behaviour. The overall productivity, total production time and descriptive statistics obtained for antibody and viable cell concentrations are presented in Table 6.6. It can be seen that the overall productivity attained was always greater than that dictated by the set point; rates of 0.5 and 1 μg ml⁻¹ h⁻¹ led to the same productivity. It can also be observed that, for production rates lower than 1 μg ml⁻¹ h⁻¹, an increase in production rate resulted in an increase in productivity, production time and mean values for antibody and viable cell concentrations and in a decrease in standard error and skewness. It may be noted that production rates higher than 1 μg ml⁻¹ h⁻¹ resulted in lower productivity and total production time.

6.6.2.2 Changing the pre-defined band for the G1b/D cell ratio

The process controller algorithm has been tested considering the pre-defined band for the G1b/D cell ratio to be the interval [0,6], when applying model-based control. Further investigation of the algorithm included consideration of different limits for this ratio band. The upper limit was reduced to 5, maintaining a lower limit of 0; the lower limit was increased to 1, 2 and 3, whilst keeping the upper limit at 6. Alteration of the lower limit corresponded to the culture being re-suspended when the G1b/D ratio value decreased to values lower than that limit; the restriction to integer values for DCT was still imposed.

It has been observed that decreasing the upper limit of the pre-defined ratio band to 5 had a small effect on process performance; productivity decreased by 2% relative to that obtained using a pre-defined ratio band of [0,6]. Increasing the lower ratio limit to
1 or 2 had no effect on process performance. However, a lower limit of 3 resulted in washout of the bioreactor contents.

6.6.2.3 Introducing a delay prior to resuspension

The process controller algorithm was further investigated considering a delay between the G1b/D cell ratio exhibiting a peak, for values greater than 6, and resuspension of the culture; delays of 1, 2, 3 and 4 h were considered. This allows for operational delays which may occur in a real situation, and which were not accounted for in the results presented in Section 6.4. It should be noted that, in the cases when the time of resuspension was rounded up to the next integer value due to the restriction imposed on DCT (Section 6.2.2), this resulted in an additional delay. It has been observed that a delay of 1 h prior to resuspension had a negligible effect upon process performance. However, greater delays resulted in washout of the bioreactor contents.

6.6.3 Results obtained for other case studies

All the results presented previously in this chapter were obtained considering a HF value of 70% and an initial DCT value of 50 h, as stated. The process controller algorithm was also tested at other HF/DCT pairs of values, namely, 80/65 and 77.5/45; the inoculum state was that considered for the previous case study. The viable cell concentration, viability and medium component concentrations obtained at these HF/DCT values, with no control action, were typical examples of, respectively, unrecoverable catastrophic failure and washout.

Both control strategies were considered to test the algorithm for the above case studies; $K_c$ was varied between 5 and 400 h ml mg$^{-1}$ and none of the features addressed in Sections 6.6.1 and 6.6.2 were considered. The optimal $K_c$ values were expected to be different from those defined for the 70/50 case study (Section 6.5). However, the process controller parameters were not optimised as the aim of the
investigation was to assess whether the algorithm was capable of dealing with unrecoverable catastrophic failure and/or washout.

Overall, the process controller algorithm was able to avoid unrecoverable catastrophic failure, applying either conventional or model-based control. The effect of $K_c$ upon the process was similar to that observed at the previous operational conditions. Furthermore, catastrophic failure was less frequent when applying model-based control than for conventional control, similarly to the results presented in Section 6.4.

The variation in viable cell concentration observed with no control action and that exhibited applying each control strategy, at a $K_c$ value of 40 h ml mg$^{-1}$, is presented in Figure 6.11, as an example. It can be seen that unrecoverable catastrophic failure did not occur when applying either conventional or model-based control. It can also be seen that catastrophic failure was considerably reduced when applying conventional control (Figure 6.11a). However, when applying model-based control, no catastrophic failure occurred and the process exhibited an almost periodic behaviour after $\approx 300$ h (Figure 6.11b); this resulted in a large increase in productivity relative to that attained with no control action.

Application of the process controller algorithm had limitations when dealing with washout, applying either control strategy. The control action was only capable of delaying washout slightly. Consequently, only a small enhancement in process performance was observed relative to that obtained with no control action, regardless of the $K_c$ value used.

### 6.6.4 Discussion of results

Overall, further investigation of the process controller algorithm confirmed that model-based control is more capable of enhancing process performance than conventional control. Furthermore, it has been proven that the set point and the
Development and Testing of the Process Controller Algorithm

pre-defined band for the G1b/D cell ratio used in the main results (Section 6.4) were the optimal values in order to optimise antibody productivity.

The process controller action parameters investigated when applying conventional control were sampling frequency and sampling noise. A significant decrease in process performance has been observed relative to that obtained using a sampling interval of 6 min and with no sampling noise. This was due to the potential 'correction' of the antibody production relative to the set point value being less frequent. Over- or under-production were thus emphasised, leading to catastrophic failure, for the reasons discussed in Section 6.5. Introduction of sampling noise affected process performance more than a decrease in sampling frequency, except for a 1% noise level. This means that the modification in the measured antibody concentration led to larger variations in set point error relative to those obtained with no noise. These observations are explained by the high variance of the normal distribution used to introduce noise, as discussed in Section 5.5.3.

Hidden mismatches were introduced when applying model-based control. It has been observed that this action parameter affected the process to a great extent when considering large mismatches in T_{G1a} and T_S or a mismatch in the flow of cells leaving the S phase. This was due to these mismatches resulting in the cell age distribution inferred on-line by the model being significantly different from the 'correct' one. Consequently, the G1b/D ratio was different from that obtained without the mismatches; this dictated DCT values which resulted in more catastrophic failure, leading to worse process performance.

It has been observed that the set point considered in the main results (Section 6.4) is the best in order to enhance process performance, for both control strategies. When applying conventional control, an increase in the 'desired' production rate resulted in an increase in DCT in order to increase production time within a cycle when under-production occurred; this led to glutamine exhaustion and frequent catastrophic failure, for the reasons discussed in Section 6.5. In the cycles where over-production occurred, DCT was greater than when the set point was lower, resulting in greater cell
growth. It can be concluded that these effects were balanced out for a rate of 0.5 μg ml⁻¹ h⁻¹. When applying model-based control, small production rates resulted in culture resuspension occurring earlier than would be dictated by the G1b/D cell ratio; this resulted in low cell growth and, thus, in low productivity. Therefore, it can be concluded that process performance was worse for lower set points due to the set point error affecting DCT. This detrimental effect also explains the results obtained for rates greater than 1 μg ml⁻¹ h⁻¹.

It is clear from the results obtained that the pre-defined band for the G1b/D cell ratio could be decreased to [2,5] without affecting process performance. This was due to this decrease in band width leading to negligible differences in DCT relative to those dictated by the ‘original’ pre-defined band, i.e., [0,6]. However, a lower limit of 3 resulted in washout as this value led to culture resuspension for high G1b/D cell ratio values, which frequently resulted in glutamine exhaustion in the next cycle.

When considering the ‘original’ pre-defined band for the G1b/D cell ratio, process performance was not affected by an additional 1 h delay prior to resuspension. This means that the cell age distribution and, thus, the G1b/D cell ratio did not change significantly during that time interval. However, significant differences occurred when considering greater delays as these led to lower viable cell concentrations. Although glutamine was present in excess in the medium, cell growth within a cycle was low and resulted in washout of the bioreactor contents.

It has been observed that the algorithm was capable of enhancing process performance at operational conditions which result in unrecoverable catastrophic failure with no control action. This is due to the process controller action resulting in reduction of the occurrence of catastrophic failure; this was particularly noticeable when applying model-based control, similarly to that observed at the 70/50 values (Section 6.4). However, it has been observed for both control strategies that the enhancement in process performance was small when considering HF/DCT values which lead to washout with no control action. This was due to the ‘original’ DCT value being small, not allowing for significant cell growth within a resuspension cycle.
6.7 Concluding Comments

The process controller algorithm was tested in repeated batch operation, considering conventional control and model-based control. It has been concluded that application of the algorithm resulted in enhancement of process performance for both control strategies, when employing proportional-only action. This was due to manipulation of DCT in order to reach a value at which catastrophic failure did not occur or was less frequent than with no control action. However, introduction of an integral term to the process controller action was not advantageous when applying either control strategy due to accumulation and amplification of set point errors. As a result, proportional-integral action led to unrecoverable catastrophic failure when applying conventional control and to washout for model-based control.

The highest overall productivity attained when applying model-based control was twice the value obtained for conventional control and less variation in productivity between consecutive cycles was observed. It is clear that model-based control led to greater enhancement of process performance than conventional control. A paramount finding was that there is no need to determine the set point error to enhance process performance if the G1b/D cell ratio is available. The results thus indicate that process control could be conducted uniquely based on the cell age distribution inferred on-line by the model. Further investigation of the process controller algorithm confirmed the above conclusions.

The process controller algorithm was further tested to investigate the effect of action parameters. When applying conventional control, sampling frequency was decreased to 4 h and up to 10% sampling noise was introduced to the measured outputs; in all cases, productivity decreased relative to the value attained with a sampling interval of 6 min and no sampling noise. When applying model-based control, hidden mismatches in $T_{G1a}$ and $T_S$ were introduced; process performance proved unaffected only for ±4% mismatches. Further testing of the process controller algorithm also included modifying algorithm parameters. It has been proven that the set point and the
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pre-defined band for the G1b/D cell ratio used in the main testing of the algorithm were the optimal values in order to optimise antibody productivity. Furthermore, it was concluded that introducing an additional delay prior to resuspension greater than 1 h would result in washout of the bioreactor contents. Finally, it has been observed that the algorithm was capable of enhancing process performance at operational conditions which result in unrecoverable catastrophic failure with no control action, but exhibited limitations when dealing with washout. This was observed when applying either control strategy, although model-based control led to better results than conventional control.

In conclusion, testing of the process controller algorithm in repeated batch operation was successful. Furthermore, the capability demonstrated when employing model-based control pre-empts success for the ultimate application of the MBO control strategy. The same conclusion had been drawn in Chapter 5 with respect to the model adaptor algorithm. However, the efficiency of both algorithms may be impaired if the model adaptor and the process controller are operated simultaneously, i.e., when the MBO control algorithm is employed. The methodology employed to integrate those algorithms into a MBO control algorithm and the results obtained when testing it will be presented in the next chapter.
Chapter 7

Testing of the

Model-based Observer Control Algorithm

7.1 Introduction

Application of model-based observer (MBO) control requires algorithms for both model adaptor and process controller, as stated in Section 3.2. In this work, those algorithms have been developed and tested separately; the results obtained were presented and discussed in Chapters 4 to 6. It has been concluded that the algorithms were capable of adapting the model, and controlling the process, on-line. The algorithms have subsequently been integrated into a MBO control algorithm. The results obtained when testing this algorithm will be presented and discussed in this chapter. Based on the discussions presented in Chapters 4 to 6, repeated batch operation was considered and proportional-only action was used in the model adaptor and, when necessary, in the process controller.

7.2 The MBO Control Algorithm

The purpose of a feedback MBO controller is exactly the same as that of any other feedback controller, i.e., to drive process outputs towards set points in order to meet pre-specified objectives. However, a MBO controller may use process information inferred on-line by a model to help controlling the process and may adapt that model on-line whenever necessary. Therefore, it involves the simultaneous use of a process controller and a model adaptor.

In this work, a MBO control algorithm was developed by considering the integration
of the algorithms previously developed and tested for the model adaptor and the process controller (Chapters 4 to 6); a printout of the code for the MBO control algorithm is presented in Appendix A. The block diagram referring to this testing stage is presented in Figure 7.1; this is a specific case of the generic schematic presented in Figure 3.1.

The control objective adopted was the same as when testing the process controller algorithm separately, i.e., to optimise antibody productivity, thus enhancing process performance. DCT was the process parameter selected to be manipulated (Figure 7.1); the additional information provided on-line by the model was the ratio between the fraction of cells in G1b and the fraction of cells in D. A selection of the model parameter/measured output combinations presented in Table 4.2 was considered, which is representative of the whole range of results previously obtained (Section 5.3). All the assumptions made in Sections 4.2 and 6.2 for the action of, respectively, the model adaptor and the process controller are still valid here.

Integration of the algorithms for the model adaptor and the process controller connects the feedback loops for the model and the process, introducing a ‘figure-of-eight’ loop to the system, as can be seen in Figure 7.1. The algorithm integration may thus result in the feedback loops affecting each other. The potential interaction between these loops may result in re-tuning the parameters of the model adaptor and/or the process controller.

7.3 Testing of the MBO Control Algorithm

The MBO control algorithm was investigated in repeated batch operation for an initial viable cell concentration of $0.09 \times 10^{10}$ cell ml$^{-1}$ and an initial glutamine concentration of 0.1 mg ml$^{-1}$. Two case studies were considered: one at the HF/DCT values of 80/50 and another at 70/50, both over 100 resuspension cycles. These were the conditions previously selected for testing the model adaptor and the process controller.
algorithms. The results obtained when testing the MBO control algorithm will thus enable assessing whether the potential interaction between the feedback loops occurs.

The process was simulated and controlled on-line and the model adapted on-line; a sampling frequency of 6 min was used. Further investigation of the MBO control algorithm covered the action parameters considered when using the algorithms separately (Sections 5.5.2 and 6.6.1), i.e.: sampling frequency, sampling noise and hidden mismatches. Furthermore, the algorithm was also tested when randomly modifying HF or DCT at the beginning of each resuspension cycle, except the first one. Finally, multiple action parameters, greater initial parameter mismatches and modifications to the operational conditions were considered in order to fully assess the capability of the algorithm to enhance process performance.

7.3.1 Simulations set up

The model parameter/measured output combinations considered for testing the MBO control algorithm are summarised in Table 7.1. It can be seen that only three of the combinations previously investigated (Table 4.2) were considered. These choices were based on the fact that the model adaptation results obtained for these scenarios covered the whole spectrum of results obtained when testing the model adaptor algorithm (Section 5.3). The model parameters were considered to be initially 10% lower (8% for TG1a) than those used to simulate the process. Initial mismatches of +10% (or +8%) were not considered at this stage as that study had already been completed when testing the model adaptor algorithm (Sections 4.4 and 5.3).

When testing the process controller algorithm, the set point had been defined based on the objective of optimisation of antibody productivity (Section 6.2.2); the measured output selected was thus antibody concentration. It can be seen in Table 7.1 that R_{Glut} and T_{Glut} were adapted using viable cell concentration as measured output. Therefore, for those simulations, the process output required for the model adaptor and the process controller actions were different, which constitutes no major alteration of the
algorithm. However, changes in antibody concentration can be related to changes in viable cell concentration and, thus, a second set point could easily be defined. This additional set point was a changing viable cell concentration equivalent to an overall cell growth rate of $5 \times 10^{-03}$ cell ml$^{-1}$ h$^{-1}$. This value corresponds to an average ratio between antibody and viable cell concentrations of $10^{-07}$ mg cell$^{-1}$ and was based on the values observed in previous simulations. Only viable cell concentration was used as measured output for both the model adaptor and the process controller when considering the viable cell set point. The aim of using two set points when adapting $R_{Glu}$ and $T_{Gla}$ was to investigate whether using different measured outputs for the model adaptor and the process controller would significantly affect model adaptation and/or process performance.

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Initial mismatch</th>
<th>Measured outputs for model adaptor</th>
<th>Measured outputs for process controller</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti Cell</td>
<td>Anti Cell</td>
</tr>
<tr>
<td>$R_{Glu}$</td>
<td>-10 %</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>$R_{Anti}$</td>
<td>-10 %</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>$T_{Gla}$</td>
<td>-8 %</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

Table 7.1 - Scenarios chosen for testing the MBO control algorithm. (Anti and Cell are the antibody and viable cell concentrations, respectively.)

Proportional-only action was employed in the model adaptor; proportional-integral control action was not considered based on the results obtained when testing the model adaptor algorithm (Chapters 4 and 5). The proportional gains ($K_a$) were the optimal values previously determined for repeated batch operation (Section 5.3), i.e.: $3 \times 10^{-13}$ mg ml$^{-1}$ cell$^2$ and $6 \times 10^{-05}$ ml h$^{-1}$ cell$^{-1}$ for adaptation of $R_{Glu}$ and $R_{Anti}$, respectively. Adaptation of $T_{Gla}$ was investigated at a $K_a$ value of $-1.7 \times 10^{-05}$ h ml cell$^{-1}$ as, when testing the model adaptor algorithm, this $K_a$ value led to the best performance measures, although $T_{Gla}$ could not be adapted.

Set point error was not determined as it had been concluded when testing the process controller algorithm that process performance was better when DCT was manipulated.
only with respect to the G1b/D cell ratio (Section 6.5); no $K_c$ values were thus considered. However, a brief study of the effect of $K_c$ was also conducted to verify the above statement when testing the MBO control algorithm. When using the viable cell set point, $K_c$ was broadly optimised in order to re-tune the process controller.

As stated in Section 7.2, the potential interaction between the feedback loops may result in re-tuning the model adaptor and/or the process controller; in other words, in re-tuning $K_a$ and/or $K_c$. It has been assumed that if interaction occurs, it will suffice to re-tune one of the gains.

### 7.3.2 Analysis of results

The analysis of results conducted corresponded to an overlap of the analyses detailed in Sections 4.3.2 and 6.3.2. Therefore, the following were determined:

- performance measures for both model and parameter errors;
- productivity attained and respective production time; and
- descriptive statistics for the distribution of values of all measured outputs and of set point error (if applicable).

The criterion adopted for successful application of the MBO control algorithm was the optimisation of process performance. Similar to when testing the process controller algorithm, this was considered to be equivalent to maximising the overall productivity, whilst minimising variations in productivity between consecutive resuspension cycles.

### 7.4 Results

An extract of the parameter error obtained when manipulating $R_{\text{Glu}_t}$, employing the MBO control algorithm and the model adaptor algorithm, is presented in Figure 7.2. It can be seen that the MBO control algorithm resulted in more oscillations in
parameter error than the model adaptor algorithm. Those oscillations are more
damped when operating at a HF/DCT pair of values of 80/50 until \( \approx 150 \) h, but
less damped thereafter. It can also be seen in Figure 7.2 that the overshoot, cross time
and settling time obtained with the MBO control algorithm are the same for both case
studies considered, but greater than those obtained when testing the model adaptor
algorithm. The cross time and settling time determined for parameter error, for both
case studies, are presented in Table 7.2 (Page 118). It may be noted that parameter and
model errors were only proportional within each resuspension cycle due to the
assumption of ‘bump-free’ operation for the model parameter value, as detailed in
Section 4.2.2.

The overall productivity and production time obtained when manipulating \( R_{\text{Glut}} \), for
both case studies, are also presented in Table 7.2. It can be seen that the process
performance obtained for the 80/50 case study was the same as that observed with no
process controller action. For the 70/50 case study, a decrease of 0.8\% in productivity
was observed relative to the value attained when testing the process controller
algorithm without the initial mismatch in \( R_{\text{Glut}} \) (Section 6.4.2). The variation in viable
cell concentration, viability, antibody concentration, set point error and DCT obtained
with and without the mismatch are presented in Figure 7.3. It can be seen that there
are significant differences between the values obtained with and without the initial
mismatch until the settling time, i.e., whilst a mismatch persists. Thereafter, the
differences become smaller and are non-existent after \( \approx 1200 \) h (28 cycles).

The performance measures obtained when manipulating \( R_{\text{Anti}} \) were the same as those
defined previously when testing the model adaptor algorithm (Table 5.3), regardless
of the case study considered. The descriptive statistics for the measured outputs and
set point error were the same as those obtained when testing the process controller
algorithm (Table 6.2). Furthermore, overall productivity and production time were
unaffected by the initial mismatch in \( R_{\text{Anti}} \), for both case studies.

Model adaptation proved successful when manipulating \( T_{\text{Glut}} \) for both case studies,
which had not been observed when separately testing the model adaptor algorithm (Section 5.3). The adaptation results were worse for the 70/50 case study than for 80/50, although cross time was smaller in the former case. An extract of the performance measures obtained for parameter error, for both case studies, are presented in Table 7.2.

The values for overall productivity and production time obtained for both case studies are also presented in Table 7.2. It can be seen that the productivity obtained for the 80/50 case study was the same as that attained with no process controller action. The best process performance observed for the 70/50 case study was achieved when DCT was manipulated with respect both to the Glb/D cell ratio and to set point error; the antibody set point was used and the optimal $K_c$ value was determined to be 40 h ml mg$^{-1}$. A 2.5% decrease in productivity was observed relative to that obtained with no initial mismatch (Table 7.2). It may be noted that a 3.3% decrease was observed for $K_c$ values lower than, or equal to, 30 h ml mg$^{-1}$, i.e., for the $K_c$ range which led to no effect of set point error upon DCT (Section 6.5).

As stated in Section 7.3.1, $R_{Glut}$ and $T_{G1a}$ were also adapted using a viable cell set point; this resulted in re-tuning the process controller. It has been observed that the best process performance when manipulating $R_{Glut}$ was obtained when the set point error had no effect on DCT; this was achieved at $K_c$ values lower than, or equal to, $1.7 \times 10^{-08}$ h cell ml$^{-1}$, for both case studies. When manipulating $T_{G1a}$, the best results were observed at a $K_c$ value of $1.7 \times 10^{-07}$ h cell ml$^{-1}$, regardless of the case study considered. At this $K_c$ value, DCT was manipulated with respect both to the Glb/D cell ratio and to set point error. This value was thus regarded as the optimal $K_c$ value, although only a broad optimisation was conducted, as stated previously (Section 7.3.1).

The performance measures obtained when manipulating $R_{Glut}$ were the same as those observed when using the antibody set point, for both case studies; the descriptive statistics obtained for the distribution of all measured outputs were also unaffected. Similarly, the set point had no effect on the system when manipulating $T_{G1a}$ for the
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<table>
<thead>
<tr>
<th></th>
<th>80/50 case study</th>
<th>70/50 case study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base case</td>
<td>Initial mismatch in</td>
</tr>
<tr>
<td></td>
<td>$R_{Glut}$</td>
<td>$T_{Glut}$</td>
</tr>
<tr>
<td>Overall productivity (µg mL⁻¹ h⁻¹)</td>
<td>1.02</td>
<td>1.02</td>
</tr>
<tr>
<td>Total production time (h)</td>
<td>5000</td>
<td>5000</td>
</tr>
<tr>
<td>Performance measures</td>
<td>$t_c$ (h)</td>
<td>25.5 (19.3)</td>
</tr>
<tr>
<td></td>
<td>$t_s$ (h)</td>
<td>326 (140)</td>
</tr>
</tbody>
</table>

- the values in brackets are the performance measures obtained when testing the model adaptor algorithm (Table 5.1).

Table 7.2 - Productivity, production time and performance measures determined when considering different initial parameter mismatches, for both case studies. (The base case for the 80/50 case study refers to no control action; for the 70/50 case study, it refers to the best values obtained when testing the process controller algorithm - Table 6.2.)

$^1$ - $t_c$: time required to reach the desired value for the first time (h).
$t_s$: settling time: time required to reach and remain at the desired value (h).
80/50 case study. However, for the 70/50 case study, model adaptation was worse than when using the antibody set point; cross time was the same, but both settling time and overshoot were larger. Furthermore, a 0.5% decrease in productivity was observed relative to the value attained using the antibody set point.

The MBO control algorithm was also tested using initial parameter mismatches greater than those in Table 7.1. The model parameters were initially lower than those used to simulate the process; only the antibody set point was used due to the above observations.

Both model adaptation and process performance were unaffected when manipulating $R_{Glu}$ for initial mismatches lower than -30%, for both case studies. An initial mismatch of -30% led to an increase of 10% in settling time and to a decrease of 2% in productivity, for the 70/50 case study; to a 28% increase in settling time and a 3% decrease in productivity, for the 80/50 case study. It has been observed for both case studies that the initial mismatch in $R_{Ant}$ could be increased to -80% without any effect on parameter error; greater mismatches led to impossible model adaptation. Process performance was the same as that observed with no mismatch, regardless of the initial mismatch introduced. Adaptation of $T_{Glu}$ was only successful for initial mismatches lower than, or equal to, -20% although the performance measures were worse than those obtained using an initial mismatch of -8%. Decreases of 4 and 36% in productivity were observed for the 70/50 and 80/50 case studies, respectively, when using an initial mismatch of -20%.

### 7.5 Discussion of Results

It is clear from the above results that application of the MBO control algorithm was successful for both case studies considered. All model parameter/measured output combinations explored led to successful adaptation, for both case studies. As the results obtained for these combinations have previously shown to cover the whole
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spectrum of results (Section 5.3), it can be concluded that the application of MBO control would be successful for all the combinations presented in Table 4.2.

The overall productivity attained in open-loop, i.e., with neither process controller nor model adaptor action, was 0.630 and 1.02 mg ml\(^{-1}\) h\(^{-1}\) for the 70/50 and the 80/50 case studies, respectively. For the former case study, productivity was increased to 1.20 mg ml\(^{-1}\) h\(^{-1}\) with process controller action when manipulating DCT with respect to the G1b/D cell ratio (Section 6.4.2). No process controller action was imposed on the latter case study as periodic behaviour was exhibited at those operational conditions (Figure 3.7); overall productivity was high and the variation in productivity between consecutive cycles was negligible. When testing the MBO control algorithm for the 80/50 case study, process performance was the same as that observed with no control action, as can be seen in Table 7.2. For the 70/50 case study, small decreases in productivity were observed relative to that obtained when testing the process controller algorithm. However, the lowest productivity attained corresponds to a 86% increase relative to that obtained in open-loop.

It has been observed that integration of the model adaptor and process controller algorithms led to interaction between process and model feedback loops for all combinations explored, except when adapting R\(_{\text{Anti}}\). However, the loop interaction only affected process performance when adapting R\(_{\text{Glut}}\) or T\(_{\text{Gia}}\) for the 70/50 case study. Furthermore, the decrease in productivity observed in these cases was small, as can be seen in Table 7.2. The effect upon process performance was due to DCT being manipulated with respect to an ‘incorrect’ prediction of the G1b/D cell ratio whilst a mismatch was present. Therefore, process performance was not affected by the presence of an initial mismatch in R\(_{\text{Anti}}\) as the settling time was very small (Table 5.3). The effect on process performance was greater when adapting T\(_{\text{Gia}}\) than when adapting R\(_{\text{Glut}}\) due to greater settling times (Table 7.2) and more oscillations for the former model parameter. This was expected as it had been previously shown that T\(_{\text{Gia}}\) directly affects model structure (Section 4.5.2).

For the 80/50 case study, no effect upon process performance was observed. This
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means that the initial parameter mismatches were not enough to result in significant changes in the Glb/D cell ratio relative to that obtained without the mismatches; any differences in DCT were thus negligible. It should be noted that these HF/DCT values resulted in periodic behaviour with no control action (Figure 3.7), whereas high occurrence of catastrophic failure was observed with no control action at 70/50 (Figure 3.9). This resulted in the system being more sensitive to process/model mismatches for the 70/50 case study.

The interaction between process and model loops affected model adaptation for both case studies, except when adapting R_{Anti}. This was due to the mismatches in R_{Glut} and T_{Gl1a} resulting in changes in DCT which, in turn, dictated different model adaptor action. Performance measures were affected much less for the 80/50 case study as the effect on DCT was negligible, as discussed above.

It can be concluded from the above discussion that integration of the model adaptor and process controller algorithms resulted in the feedback loops affecting each other, except when adapting R_{Anti}. However, the interaction of the feedback loops proved very small and no re-tuning of K_a or K_c was required, except when adapting T_{Gl1a} for the 70/50 case study. In this case, K_c was increased from 30 to 40 h ml mg^{-1}; at this value, the set point error affects DCT, as discussed in Section 6.5. The determination of set point error proved advantageous in this case as it resulted in earlier resuspension within a cycle and, thus, in less subsequent catastrophic failure relative to that dictated by the wide pre-defined Glb/D ratio band.

It has been observed that using an antibody set point or a viable cell set point had no effect on the results obtained, for both case studies. The set point error affected DCT when manipulating T_{Gl1a}, but not when manipulating R_{Glut}, regardless of the set point used. This was expected and is due to the interdependency between those outputs and, thus, the set points. The difference in productivity and performance measures observed when using the different set points, for adaptation of T_{Gl1a}, was minimal and may be attributed to the broad optimisation of K_c.
Overall, greater initial parameter mismatches had the same effect on model adaptation as when testing the model adaptor algorithm; this has been discussed in detail in Section 4.5.3. Model adaptation proved impossible when using large initial mismatches, for all model parameters. This was due to significant differences between the cell age distributions for the process and the model, at those large mismatch values. It has been observed that the better the performance measures when using a -10% (or -8%) initial mismatch, the more that mismatch could be increased without affecting process performance significantly. This was expected as better performance measures correspond to the G1b/D cell ratio being corrected within a shorter period of time.

It should be noted that, when testing the model adaptor algorithm, 'mirrored' behaviour for parameter (and model) error was only observed for ±10% initial mismatches. For greater parameter mismatches, performance measures were always better for the positive mismatches (Section 4.4.2.1). Therefore, the testing of the MBO control algorithm was conducted at the 'worst' conditions. It can thus be concluded that the algorithm is not only capable of enhancing process performance using the negative initial parameter mismatches considered above, but it would also be capable of dealing with the equivalent positive mismatches.

### 7.6 Further Investigation

The MBO control algorithm was further tested to investigate the effect upon both process performance and model adaptation of modifying action parameters. The parameters addressed were those considered when separately testing the algorithms for the model adaptor (Section 5.5.2) and the process controller (Section 6.6.1), i.e., sampling frequency, sampling noise and hidden mismatches. Sampling frequency and noise affect the measured output and, thus, the value of model error and set point error. Sampling noise was only added to the measured outputs used for the process
controller action when manipulating \( T_{Gia} \) as the set point error has no effect on the value of DCT for the other model parameters considered (Section 7.4).

Further investigation of the MBO control algorithm also included addressing the effect of randomly modifying HF or DCT at the beginning of each resuspension cycle, except the first one. It has been shown in Section 3.5.3 that small variations in either HF or DCT may result in different process behaviour and, thus, different process performance. However, small variations in operational conditions may occur in a real situation, mainly due to human error.

Finally, combination of the above features and introduction of greater initial mismatches have been considered to simulate a 'worst scenario' situation. The results obtained will enable to better assess the applicability of the MBO control algorithm.

It may be noted that the viable cell set point has not been used when further investigating the algorithm as it has been concluded that the results obtained were independent of the set point used (Section 7.5). An overview of the results obtained will be presented in Sections 7.6.1 to 7.6.3, according to the features investigated, and discussed in Section 7.6.4. Unless otherwise stated, it should be assumed that the results were obtained at the optimal \( K_a \) values and not considering set point error to manipulate DCT, except for \( T_{Gia} \).

### 7.6.1 Results obtained when modifying action parameters

#### 7.6.1.1 Decreasing sampling frequency

The measured outputs used to calculate the model error were considered to be sampled at intervals of 1, 2 and 4 h; the latter value had not been considered when investigating the model adaptor algorithm (Section 5.5). It has been observed that decreasing sampling frequency affected model adaptation, but not process performance, for both case studies considered. \( K_a \) had to be decreased in order to
obtain the same performance measures as when using a sampling interval of 6 min. However, adaptation of $R_{\text{Glut}}$ was not successful for sampling intervals greater than 2 h, regardless of the $K_a$ value.

### 7.6.1.2 Introducing sampling noise

Sampling noise was introduced to the measured outputs at levels of 1, 5 and 10%; these were the same values as those considered when separately testing the model adaptor and the process controller algorithms (Sections 5.5.2.2 and 6.6.1.2). Overall, introduction of sampling noise affected model adaptation, but the effect on process performance was negligible, regardless of the case study considered. In general, adaptation only proved successful at 1% noise, despite the decreases in $K_a$.

### 7.6.1.3 Introducing a hidden mismatch

The hidden mismatches previously introduced for testing the model adaptor and the process controller (Section 5.5.1) were also considered for testing the MBO control algorithm; adaptation of $T_{\text{Gl}}$ was not considered, for the reasons detailed in Section 5.5.1. Overall, introduction of a hidden mismatch had a greater effect for the 70/50 case study than for 80/50. Sub-optimal $K_a$ values were used in order to obtain similar performance measures as those observed without any mismatch. However, adaptation proved impossible when introducing a +100% mismatch in $T_{\text{Gl}}$, for manipulation of $R_{\text{Glut}}$ and when introducing a -100% mismatch in $T_{\text{Gl}}$ or $T_s$, or the flow mismatch, for all model parameters. It has been observed that process performance was only affected when considering -100% mismatches in $T_{\text{Gl}}$ or $T_s$ or when altering the cell flow. In general, manipulation of $R_{\text{Glut}}$ led to better adaptation than $R_{\text{Anti}}$, for all the mismatches considered. It has also been observed that adaptation was less sensitive to mismatches in $T_{\text{Gl}}$ than in $T_s$.

Further investigation of the MBO control algorithm also included introducing two other hidden mismatches. These consisted of altering the value of $R_{\text{Glut}}$ and $S_{\text{Max}}$ when manipulating $R_{\text{Anti}}$ and $R_{\text{Glut}}$, respectively. The former parameters were both altered by
±5 or ±10% relative to the values used to simulate the process (Table 4.1). It has been observed that the system was less affected by a hidden mismatch in $R_{Glu}$ than in $S_{Max}$, for both case studies. However, it should be noted that the results obtained for the 70/50 case study were undesirable, both in terms of model adaptation and process performance. For the 80/50 case study, a negligible effect was observed in the values of performance measures, descriptive statistics and productivity for any of the hidden parameter mismatches introduced.

### 7.6.2 Results obtained when modifying the operational conditions

The operational conditions, i.e., HF and DCT, were separately altered at the beginning of each resuspension cycle, except the first one. The modification introduced consisted of randomly changing one of those values within a defined range of values; the maximum band considered was ±100%.

It has been observed that adaptation of $T_{Glu}$ was impossible in all cases, whereas adaptation of $R_{Anti}$ was unaffected even when modifying HF or DCT within a band of ±100%. Adaptation of $R_{Glu}$ was also successful in all cases, but the performance measures were worse than those obtained at the ‘correct’ operational conditions (Section 7.4).

Process performance was less affected by modifications in DCT than in HF, for all model parameters and both case studies. It has also been observed that process performance was more affected by modifying operational conditions when adapting $T_{Glu}$; a decrease in productivity of ≈20% was observed when altering either HF or DCT by ±5%. No effect on process performance was observed when adapting $R_{Anti}$ and altering DCT, for either case study, although modifying HF by ±5% led to a 9% decrease in productivity. When adapting $R_{Glu}$, productivity was unaffected when altering DCT for the 80/50 case study and decreased by 5% for the 70/50 case study, regardless of the modification introduced. Altering HF within a band of ±5% led to
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decreases of 10 and 61% in productivity for the 70/50 and 80/50 case studies, respectively.

7.6.3 Results obtained when introducing multiple challenges to the algorithm

Combinations of the features addressed in Sections 7.6.1 and 7.6.2 were considered to further test the MBO control algorithm. Greater initial parameter mismatches were also used to further the challenge.

Model adaptation proved impossible for all challenges considered when manipulating any of the model parameters, regardless of the case study. Process performance was poor and undesirable productivity values were obtained when manipulating T_{G1a}, for both case studies, and when manipulating R_{Glut}, for the 70/50 case study. However, when manipulating R_{Glut}, for the 80/50 case study, productivity remained the same as that obtained with no control action even when considering 4 h sampling intervals, 10% sampling noise, -4% hidden mismatch in T_{G1a} and -20% initial parameter mismatch in R_{Glut}. The limits of the algorithm were reached when randomly modifying HF or DCT at the beginning of each cycle within a band of ±10% in addition to the above features; productivity decreased by 90% in both cases. The viable cell concentration and viability obtained when modifying HF are presented in Figure 7.4, as an example. It can be seen that randomly changing HF at the beginning of each resuspension cycle led to washout of the bioreactor contents, destroying the periodicity exhibited with no control action and when considering the previous features.

It has also been observed that productivity was unaffected when manipulating R_{Anti}, considering 4 h sampling intervals, 10% sampling noise, -4% hidden mismatch in T_{G1a} and -80% initial parameter mismatch in R_{Anti}. In other words, the productivity was that attained with no control action, for the 80/50 case study, and that attained with process controller action, for the 70/50 case study (Section 7.5). Modifying DCT
by any value in addition to the above features had no effect on productivity, regardless of the case study. However, modifying HF by ±10% led to a decrease in productivity of over 90%, for both case studies. The viable cell concentration and viability obtained for the 70/50 case study are presented in Figure 7.5. It can be seen that randomly modifying HF resulted in washout of the bioreactor contents very early in the simulation.

7.6.4 Discussion of results

Overall, model adaptation has proven more sensitive to the features addressed when further investigating the MBO control algorithm than process performance. This was expected from the results obtained when separately testing the model adaptor and the process controller algorithms (Sections 5.5 and 6.6).

As discussed in Section 7.5, the interaction between the feedback loops for the process and the model is due to incorrect prediction of the G1b/D cell ratio whilst there is a mismatch. It has been observed when further investigating the algorithm that, in some cases, model adaptation was impossible but the productivity was maintained at the values attained without the initial mismatch. This was due to considerable mismatches in some model parameters resulting in changes in the G1b/D cell ratio which affect DCT in the same way as if there was no mismatch. It is, thus, a consequence of the pre-defined G1b/D ratio band being wide.

Investigation of the effect of modifying action parameters upon model adaptation and process performance was similar to that conducted when separately testing the algorithms for the model adaptor and the process controller and yielded similar results. Detailed analysis of those results can be found in Sections 5.5.3 and 6.6.4. However, two additional hidden mismatches were introduced to $R_{\text{Glut}}$ and $S_{\text{Max}}$; these parameters are not related to the model structure, whereas the previously introduced hidden mismatches directly affected the model structure.
It has been observed that a hidden mismatch in $R_{Glut}$ affected the system less than a mismatch in $S_{Max}$. This was due to $R_{Glut}$ not having a direct effect on $R_{Anti}$, whereas co-variance exists between $R_{Glut}$ and $S_{Max}$, as observed previously (Faraday, 1994).

It has also been observed that the results obtained for the 80/50 case study were better than those obtained when altering model parameters directly related to the model structure, although that was not always the case for the 70/50 case study. It can thus be concluded that altering parameters not related to the model structure has a smaller effect on process performance when using operational conditions which lead to periodic behaviour with no control than when aperiodic behaviour is exhibited.

The effect on model adaptation observed when randomly modifying HF or DCT was greater when manipulating $T_{Gla}$ than $R_{Glut}$ and no effect was observed when manipulating $R_{Anti}$. The same 'gradation' of effects had been observed in the main results (Section 7.4) and the same explanation applies (Section 7.5).

Randomly modifying the value of DCT at the beginning of each resuspension cycle had no effect on process performance when manipulating $R_{Glut}$ or $R_{Anti}$. This was due to DCT being the manipulated process parameter; thus, 'errors' on its value were quickly eliminated. The decrease in productivity observed when manipulating $T_{Gla}$ was due to the large settling time observed, which led to DCT being manipulated with respect to an 'incorrect' G1b/D cell ratio for a long period of time. Therefore, the 'error' in DCT introduced at the beginning of each cycle could not be eliminated by the process controller action whilst a mismatch was present. Modifying HF had a greater effect on process performance than altering DCT as the process controller was unable to manipulate the former. Instead, modifications in HF interfered with model adaptation and, consequently, resulted in the process controller dictating DCT values different from those observed at the 'correct' HF value. This was particularly noticeable when manipulating $T_{Gla}$ due to the large settling time and, thus, to the prolonged interaction between process and model loops.

In a real situation, an operator could accidentally alter the value of HF or DCT, or both. It may be noted that, in open-loop, ±5% variations in HF or DCT lead to
decreases in productivity of 2 or 82%, respectively, for the 70/50 case study; to decreases of 16 or 51%, respectively, for the 80/50 case study. It can thus be concluded from the results obtained that the MBO control algorithm was capable of reducing or eliminating the effects observed in open-loop, which confirms the robustness of the algorithm developed.

The final test for the MBO control algorithm consisted of modifying multiple action parameters, modifying operational conditions and using greater initial parameters mismatches. Although adaptation was impossible in all cases, productivity was maintained at, or within, desirable values for all model parameters considered, except $T_{G1a}$, regardless of the case study. Therefore, the only limitation to the application of the algorithm developed in such ‘drastic’ conditions will be when trying to adapt model parameters directly related to the model structure. It can thus be concluded that the MBO control algorithm developed has wide potential application for the control of real bioreactors operating in repeated batch (or batch) operation.

### 7.7 Concluding Comments

The MBO control algorithm was tested in repeated batch operation, employing proportional-only action. It has been concluded that application of the algorithm was successful for both case studies as it resulted in maintaining or enhancing process performance relative to that observed with no control action. For the 70/50 case study, MBO control resulted in very small decreases in productivity relative to that obtained when testing the process controller algorithm, when adapting $R_{Glut}$ and $T_{G1a}$. As discussed in Section 5.4, batch operation results can be inferred from results obtained in repeated batch. It can thus be concluded that application of the MBO control algorithm would also be successful in batch operation.

It has been observed that integration of the model adaptor and process controller algorithms led to interaction between process and model feedback loops for all
combinations explored, except when adapting $R_{\text{Anti}}$. However, the loop interaction was, overall, small and did not result in re-tuning of $K_a$ or $K_c$. The only exception was when adapting $T_{\text{Gla}}$, for the 70/50 case study; however, the modification in $K_c$ was small. Furthermore, manipulation of DCT with respect to set point error still proved to be detrimental, except when adapting $T_{\text{Gla}}$. This is of paramount importance as it shows the advantages in using this alternative control strategy in preference to conventional control.

Further investigation of the MBO control algorithm confirmed the conclusions drawn when further testing the model adaptor and process controller algorithms. Overall, model adaptation proved more sensitive than process performance to all the challenges given to the algorithm. The greatest effect upon process performance and model adaptation was observed when simultaneously modifying combinations of action parameters, randomly modifying HF or DCT and using greater initial mismatches. Model adaptation proved impossible in all of these cases; process performance was undesirable only when adapting $T_{\text{Gla}}$, for both case studies, and when adapting $R_{\text{Glut}}$ for the 70/50 case study.

When adapting $R_{\text{Glut}}$, for the 80/50 case study, the limits of the algorithm were reached when modifying HF or DCT within a band of ±10% in addition to considering 4 h sampling intervals, 10% sampling noise, -4% hidden mismatch in $T_{\text{Gla}}$ and -20% initial parameter mismatch in $R_{\text{Glut}}$. When adapting $R_{\text{Anti}}$, the limits of the algorithm were reached when modifying HF within a band of ±10% in addition to considering 4 h sampling intervals, 10% sampling noise, -4% hidden mismatch in $T_{\text{Gla}}$ and -80% initial parameter mismatch in $R_{\text{Anti}}$. The MBO control algorithm developed has thus proven robust in simulation under a wide range of conditions. It can be concluded from these results that the algorithm has great potential applicability in bioreactor control.
Chapter 8
Closing Discussion and Conclusions

8.1 Closing Discussion

The application of model-based observer (MBO) control to bioreactors has been proposed in this dissertation. This control strategy has been used to enhance antibody productivity and, thus, process performance in a particular system. Although MBO control has been successfully applied to chemical processes (Jones and Gawthrop, 1992; Tong and Bobis, 1993; Gawthrop and Ponton, 1996), no communications were found in the literature on its application to bioreactors; this work thus constitutes a novel application of MBO control.

Bioreactor operation may result in the acquisition of a great amount of representative data, although these are usually obtained by off-line techniques, as stated in Chapter 2. Some bioreactors can be successfully monitored and controlled in this way (Agrawal, 1989; Chang and Lim, 1990; Ramseier et al., 1993). However, that data often lacks important process information which, if available, could lead to better bioreactor control.

The alternative approach explored in this dissertation is to employ a model to estimate, on-line and continuously, key process variables; to feed this information to a controller in order to enhance process performance; and to adapt the model whenever necessary to ensure it remains a good representation of the process. Estimation of cell age distribution has proven to be a means of understanding the underlying dynamics of the process and, in terms of the G1b/D cell ratio, it is a good indicator of catastrophic failure. In the particular case of the mm321 hybridoma cell line, antibody is produced in the G1b and S phases at a fixed rate, $R_{Anti}$. It is clear that the greater the percentage of cells which is in those phases, the greater the overall antibody
production; monitoring of the G1b/D cell ratio provides an indication of whether glutamine is being consumed effectively. The next stage will thus be to use cell age distribution to anticipate process behaviour and act on the process in order to increase the percentage of cells in the G1b and S phases without leading to glutamine exhaustion.

The work presented in this dissertation focused on a particular system and required system-specific information; the control perspective adopted is hence a physical-model based control (PMBC) approach (Costello and Gawthrop, 1997). However, the specific model for the mm321 hybridoma cell line used has most features of the generic model CELCYMUS. Furthermore, model adaptation was conducted manipulating parameters in order to fully explore the whole dynamic range of the model. In addition, the methodology employed to develop the control algorithms for the process controller and model adaptor, and their subsequent integration into an overall MBO control algorithm is generic in nature. It can thus be concluded that, in principle, the MBO control algorithm developed could be successfully applied to any other specific model built within CELCYMUS or, in fact, any other structured, segregated model.

The above observations do not suggest that investment should not be made in the development of on-line monitoring sensors and techniques. In fact, this could be conducted in parallel with the development of estimator models; complementary application could contribute to improving both models and measuring techniques, which in principle would be beneficial for the monitoring of process outputs required for model adaptation. However, it seems more economical to control bioreactors based on model estimation rather than on-line measurements as modelling is mainly dependent on the ability of the modeller to translate the process mechanism into a representative set of equations. This approach is supported by the advances in computational power which enable inexpensive, fast and accurate solution of complex models, as well as simpler process descriptions. In this work, control of a 5000 h repeated batch run, considering a 6 min integration step length, was achieved in
Closing Discussion and Conclusions

≈10 min, using a Pentium-I personal computer, at 150 MHz; this was reduced to less than 1 min, on average, using a super computer.

Separately testing the algorithms for the model adaptor and the process controller has provided a good indication of the results obtained with the MBO control algorithm as the interaction between process and model feedback loops proved minimal or non-existent. The exception was when manipulating model parameters directly related to the model structure as these could be adapted when employing the MBO control algorithm, but not when testing the model adaptor algorithm. This demonstrates the robustness of the MBO controller (Signal and Lee, 1992). The performance measures obtained could be further improved by manipulating those model parameters together with some other parameter not related to the model structure.

The approach adopted in this work has highlighted the importance of several features for bioreactor operation and control. In general, no effect of sampling frequency and noise was observed upon process performance as DCT was manipulated only with respect to the Glb/D cell ratio inferred on-line by the model. However, model adaptation could not cope with the presence of noise greater than 1%, despite the effect of sampling frequency being negligible. The implication of this is that, when applying MBO control to a real bioreactor, preventive measures should be taken, such as filtering and signal smoothing, in order to reduce the effect of sampling noise and ensure continuing successful model adaptation as 1% noise is an unrealistic value. However, the process outputs used to determine model error and hence required for model adaptation can be sampled at 4 h intervals without any detrimental effect on the performance measures. Therefore, these outputs can be measured by off-line, reliable techniques instead of money and effort having to be put into developing or improving measuring sensors and techniques.

Another important feature highlighted in this work was the presence of hidden mismatches, which would inevitably occur in a real situation, and which affected both process performance and model adaptation for mismatches greater than 4%. This
implies that success of the application of MBO control to bioreactors is dependent on the presence of a robust model. This can only be achieved with the use of structured, segregated models. ANN, fuzzy or ‘grey-box’ models, although being currently often thought of as a panacea, cannot contribute significantly to efficient bioreactor control as they provide no understanding of process mechanisms. Therefore, these would have proven even more susceptible to hidden mismatches.

Finally, the influence of human error on process behaviour was also analysed by randomly altering DCT or HF at the beginning of each resuspension cycle. Although model adaptation was affected in both cases, productivity only decreased when modifying HF. As concluded before, this was due to DCT being the manipulated process parameter and, thus, any errors being corrected quickly. This has very important practical consequences for the implementation of MBO control to a real bioreactor as it indicates that the manipulated process parameter should be a variable which is prone to ‘uncontrollable’ modifications by human error.

The conclusions drawn from this research work indicate that implementation of MBO control to bioreactors and the biotechnology industry in general has great and wide applicability. The use of models built within CELCYMUS for tumor research has already been suggested (Faraday, 1994). Incorporation of such models within a MBO control algorithm could be useful for the development of effective cancer treatment techniques. Other potential applications of the work presented in this dissertation include increasing the purity and production quantity of various medicines or controlling the alcohol content in drinks such as beer. In all of these cases, strict control requirements such as Food and Drug Administration regulations have also to be taken into account, which were not addressed in this work.

It has been stated (Sanders, 1998) that although many advanced control strategies work well in simulation, their implementation into a real operating environment may prove unsuccessful for a variety of reasons. In fact, most of the features suggested by Sanders as responsible for the difficulties when implementing advanced control strategies (Sanders, 1998) have been considered in this work, which constitutes
another incentive for the application of the MBO control algorithm developed to the control of a real bioreactor.

Monitoring and modelling aspects have been considered in this work by modifying sampling frequency, introducing noise and hidden mismatches. Furthermore, operator error has also been considered by randomly modifying operational conditions at the beginning of the resuspension cycles. Changes in operational range have also been considered as different HF/DCT values were considered throughout the work. It has been observed that the MBO control algorithm was capable of dealing with operational conditions which lead to periodic, aperiodic or unrecoverable catastrophic failure behaviour without any control action, although limitations were observed for washout. However, mechanical failures of instrumentation and introduction of sampling delays have not been addressed. Furthermore, the lack of maintenance or long-term support by strategy developers was beyond the context of this dissertation.

8.2 Overall Conclusions

The objectives of this work have been met in that:

- a control algorithm has been developed and successfully tested for the model adaptor, both in batch (Chapter 4) and repeated batch operation (Chapter 5);
- a control algorithm has been developed and successfully tested for the process controller, in repeated batch operation (Chapter 6);
- the above have been integrated into an overall MBO control algorithm, which was successfully tested in repeated batch operation (Chapter 7); and
- this overall control algorithm has led to enhancement of process performance based on the cell age distribution inferred on-line by the model, whilst adapting the model whenever necessary.
A number of observations can be made from this work:

- estimation of cell age distribution provides a good indication of the occurrence of catastrophic failure and may be used to understand the underlying dynamics of the process;

- process performance proved better when only manipulating DCT with respect to the information inferred on-line by the model, eliminating the need to monitor process outputs or for controller tuning;

- interaction between model and process loops in MBO control proved minimal or non-existent;

- the MBO control algorithm developed has proven robust for a wide range of action parameters and across a wide range of HF/DCT values, including conventional batch operation.

Finally, it can be concluded that the work presented in this dissertation pre-empts success for the application of MBO control to a real bioreactor. The potential applications areas may be as diverse as the medical field or the brewing industry. This could certainly be achieved with models developed within CELCYMUS, but the use of other structured, segregated models would, in principle, also be viable.

### 8.3 Recommendations

Several research areas have been identified to further the work presented in this dissertation. It is thus recommended that:

- other propagation methods are considered for testing the MBO control algorithm, namely of continuous operational mode;

- the effect of different feeding strategies is also investigated;

- model adaptation by manipulation of multiple model parameters is considered, for parameters either related or non-related to model structure;

- sampling delay is also considered for further investigation of the MBO control algorithm, namely of the effect upon model adaptation;
• cell age distribution is used in feedforward in order to analyse any potential benefits in terms of process performance and/or model adaptation;
• application of MBO control is attempted using other models built within CELCYMUS to verify the conclusions drawn in this work, although other structured, segregated models should also be considered for comparison purposes; and, finally,
• the work presented here is applied to a real system as the robustness of the MBO control algorithm developed has been proven.

The use of structured, segregated models to represent bioprocess behaviour has not received much attention since the 1960s; their application in bioreactor control has also been limited due to the mathematics involved in their development. The results presented in this dissertation have demonstrated that these models can be incorporated within MBO controllers. In fact, this approach has proven more efficient in enhancing process performance than conventional control strategies and has eliminated the need to determine set point error, a fundamental concept in conventional control theory. Furthermore, the presence of a structured, segregated model provided a means of understanding the underlying dynamics of the bioprocess. In conclusion, the results obtained pre-empt success for the application of MBO control to bioreactors.
**Figures**

![Diagram](image)

**Figure 3.1** - Schematic of the model-based observer (MBO) control strategy.
Figure 3.2 - Schematic of the specific model employed as an observer for the application of the MBO control strategy (Faraday, 1994 - adapted).
**Figure 3.3** - Batch operation: viable cell concentration, medium component concentrations and viability for a typical batch run.

**Figure 3.4** - Batch operation: cell age distribution relative to Figure 3.3.
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Figure 3.6 - Batch operation: cell age distribution relative to Figure 3.5.
Figure 3.7 - Repeated batch operation: viable cell concentration (●) and viability (■) for HF=80% and DCT=50 h, over 100 resuspension cycles. (Only the initial and final viable cell concentration values of each cycle are presented.)
Figure 3.8 - Repeated batch operation: cell age distribution relative to Figure 3.7.
Figure 3.9 - Repeated batch operation: viable cell concentration (●) and viability (■) for HF=70% and DCT=50 h, over 100 resuspension cycles. (Only the initial and final viable cell concentration values of each cycle are presented.)
Figure 3.11 - Repeated batch operation: viable cell concentration (♦) and viability (■) for HF=80% and DCT=65 h, over 100 resuspension cycles. (Only the initial and final viable cell concentration values of each cycle are presented.)
Figure 3.12 - Repeated batch operation: cell age distribution relative to Figure 3.11.
Figure 3.13 - Repeated batch operation: viable cell concentration (●) and viability (■) for HF=85% and DCT=50 h, over 100 resuspension cycles. (Only the initial and final viable cell concentration values of each cycle are presented.)
Figure 3.14 - Repeated batch operation: cell age distribution relative to Figure 3.13.
Figure 4.1 - Elements for the development and testing of the model adaptor algorithm (dark lines) and remaining elements of the MBO control strategy, not considered at this stage (grey lines). (The dotted lines for the disturbances refer to the discontinuous propagation methods considered.)
**Figure 4.2** - Batch operation: model error for adaptation of $R_{\text{Glu}}$ using viable cell concentration as measured output, employing proportional-only action ($K_a$ has units of mg ml h$^{-1}$ cell$^{-2}$).

**Figure 4.3** - Batch operation: parameter error for adaptation of $R_{\text{Glu}}$ using viable cell concentration as measured output, employing proportional-only action ($K_a$ has units of mg ml h$^{-1}$ cell$^{-2}$).
**Figure 4.4** - Batch operation: model error for adaptation of $R_{\text{Glut}}$ using glutamine concentration as measured output, employing proportional-only action ($K_a$ has units of ml h$^{-1}$ cell$^{-1}$).

**Figure 4.5** - Batch operation: parameter error for adaptation of $R_{\text{Glut}}$ using glutamine concentration as measured output, employing proportional-only action ($K_a$ has units of ml h$^{-1}$ cell$^{-1}$).
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**Figure 4.8** - Batch operation: model error for adaptation of $R_{\text{Anti}}$ using antibody concentration as measured output, employing proportional-only action ($K_a$ has units of ml h$^{-1}$ cell$^{-1}$).

**Figure 4.9** - Batch operation: parameter error for adaptation of $R_{\text{Anti}}$ using antibody concentration as measured output, employing proportional-only action ($K_a$ has units of ml h$^{-1}$ cell$^{-1}$).
Figure 4.10 - Batch operation: model error for adaptation of $R_{\text{Ant}}$ using antibody concentration as measured output, employing proportional-integral action ($K_a$ has units of ml h$^{-1}$ cell$^{-1}$ and $\tau_a$ has units of h).

Figure 4.11 - Batch operation: parameter error for adaptation of $R_{\text{Ant}}$ using antibody concentration as measured output, employing proportional-integral action ($K_a$ has units of ml h$^{-1}$ cell$^{-1}$ and $\tau_a$ has units of h).
**Figure 4.12** - Batch operation: model error for adaptation of $T_{Gia}$ using viable cell concentration as measured output, employing proportional-only action ($K_a$ has units of h ml cell$^{-1}$).

**Figure 4.13** - Batch operation: parameter error for adaptation of $T_{Gia}$ using viable cell concentration as measured output, employing proportional-only action ($K_a$ has units of h ml cell$^{-1}$).
Figure 4.14 - Batch operation: cell age distributions for adaptation of \( R_{\text{G4at}} \) using viable cell concentration as measured output, at the optimal \( K_a \) value, for \( \pm 10\% \) initial mismatches and cell age distribution for the process. (Data relative to the G1', G2 and M phases are not shown.)

Figure 4.15 - Extract of Figure 4.14: the initial 20 h.
Figure 5.1 - Repeated batch operation: model error for adaptation of $R_{\text{GluH}}$ using viable cell concentration as measured output, employing proportional-only action ($K_a$ has units of $\text{mg ml}^{-1} \text{h}^{-1} \text{cell}^{-2}$).

Figure 5.2 - Repeated batch operation: parameter error for adaptation of $R_{\text{GluH}}$ using viable cell concentration as measured output, employing proportional-only action ($K_a$ has units of $\text{mg ml}^{-1} \text{h}^{-1} \text{cell}^{-2}$).
Figure 5.3 - Repeated batch operation: model error for adaptation of $R_{\text{Glut}}$ using glutamine concentration as measured output, employing proportional-only action ($K_a$ has units of ml h$^{-1}$ cell$^{-1}$).

Figure 5.4 - Repeated batch operation: parameter error for adaptation of $R_{\text{Glut}}$ using glutamine concentration as measured output, employing proportional-only action ($K_a$ has units of ml h$^{-1}$ cell$^{-1}$).
Figure 5.5 - Repeated batch operation: parameter error for adaptation of $R_{\text{Anti}}$ using antibody concentration as measured output, for a $K_a$ value of $6 \times 10^{-9}$ ml h$^{-1}$ cell$^{-1}$ and considering 1% sampling noise.

Figure 5.6 - Repeated batch operation: parameter error for adaptation of $S_{\text{Max}}$ using viable cell concentration as measured output, at a $K_a$ value of $-5 \times 10^{-12}$ mg ml cell$^{-2}$ and a -10% initial parameter mismatch, for a -4% hidden mismatch in $T_{G1a}$. 
Figure 5.7 - Repeated batch operation: parameter error for adaptation of $R_{\text{Anti}}$ using antibody concentration as measured output, at a $K_a$ value of $6 \times 10^{-7}$ ml h$^{-1}$ cell$^{-1}$ and considering 1% sampling noise, for sampling intervals of 6 min and 2 h.

Figure 5.8 - Repeated batch operation: parameter error for adaptation of $S_{\text{Max}}$ using viable cell concentration as measured output, at a $K_a$ value of $-5 \times 10^{-12}$ mg ml cell$^{-2}$ and a -10% initial parameter mismatch, for a -4% hidden mismatch in $T_{\text{Glu}}$, considering 1% sampling noise, for sampling intervals of 6 min and 2 h.
Figure 6.1 - Elements for the development and testing of the process controller algorithm (dark lines) and remaining elements of the MBO control strategy, not considered at this stage (grey lines). (The dotted lines for the disturbances refer to the discontinuous propagation methods considered.)
Figure 6.2 - Viable cell concentration (●) and ratio between the fraction of cells in G1b and the fraction of cells in D (—) for HF=70% and DCT=50 h, over the initial 25 resuspension cycles. (Six cell concentration values are presented per cycle. The dotted line refers to the pre-defined upper limit for the G1b/D cell ratio.)
Figure 6.3 - (a) Viable cell concentration (●), viability (●) and (b) antibody concentration (●) obtained with no control action at HF=70% and DCT=50 h, over 100 resuspension cycles.
Figure 6.4 - Mean for all measured outputs and for set point error obtained at several $K_c$ values, when applying conventional control.
Figure 6.5 - (a) Viable cell concentration (●), viability (★); (b) antibody concentration (●); (c) set point error (●) and (d) DCT (★) obtained at a $K_c$ value of 95 h ml mg⁻¹, employing conventional control.
Figure 6.5 (continued)

(c)

(d)

Figure 6.5 (continued)
Figure 6.6 - (a) Viable cell concentration (•), viability (●); (b) antibody concentration (●); (c) set point error (●) and (d) DCT (●) obtained at a $K_c$ value of 50 h ml mg$^{-1}$, employing conventional control.
Figure 6.6 (continued)
Figure 6.7 - Mean for all measured outputs and for set point error obtained at several $K_c$ values, when applying model-based control.
Figure 6.8 - (a) Viable cell concentration (●), viability (○); (b) antibody concentration (●); (c) set point error (■) and (d) DCT (○) obtained at a $K_v$ value of 45 h ml mg$^{-1}$, employing model-based control.
Figures

Figure 6.8 (continued)
Figure 6.9 - (a) Viable cell concentration (●), viability (■); (b) antibody concentration (●); (c) set point error (■) and (d) DCT (●) obtained at a $K_c$ value of 30 h ml mg$^{-1}$, employing model-based control.
Figure 6.9 (continued)

(c)  

(d)
Figure 6.10 - Ratio between the fraction of cells in phase G1b and the fraction of cells in phase D obtained: (a) with no control action; applying model-based control, at $K_c$ values of (b) 45 h ml mg$^{-1}$ and (c) 30 h ml mg$^{-1}$. (The dotted line refers to the pre-defined upper limit for this ratio.)
Figure 6.11 - Viable cell concentration obtained for HF=80% and initial DCT=65h: with no control action (•) and at a $K_c$ value of 40 h ml mg$^{-1}$ (○), employing (a) conventional control and (b) model-based control.
known disturbance

process controller algorithm

model adaptor algorithm

process output = desired model output

set point error

model error

additional information

model output

Figure 7.1 - Block diagram referring to the testing of the MBO control algorithm.
Figure 7.2 - Parameter error obtained for adaptation of $R_{\text{Glu}}$ using viable cell concentration as measured output, employing the MBO control algorithm, for both case studies and the model adaptor algorithm.
Figure 7.3 - (a) Viable cell concentration (●), viability (●); (b) antibody concentration (●), (c) set point error (●) and (d) DCT (●) obtained for adaptation of $R_{G_{\text{Cuts}}}$ when testing the MBO control algorithm for the 70/50 case study. (The values in yellow refer to the results obtained without the initial mismatch - Figure 6.9).
Figure 7.3 (continued)
Figure 7.4 - Viable cell concentration (●) and viability (■) obtained when manipulating $R_{\text{Gut}}$ for the 80/50 case study, considering 4 h sampling intervals, 10% sampling noise, -4% hidden mismatch in $T_{\text{G1a}}$, -20% initial parameter mismatch in $R_{\text{Gut}}$ and randomly modifying HF at the beginning of each resuspension cycle within a ±10% band. (The grey values correspond to the viable cell concentration (●) and viability (■) obtained with no control action.)

Figure 7.5 - Viable cell concentration (●) and viability (■) obtained when manipulating $R_{\text{Anti}}$ for the 80/50 case study, considering 4 h sampling intervals, 10% sampling noise, -4% hidden mismatch in $T_{\text{G1a}}$, -80% initial parameter mismatch in $R_{\text{Anti}}$ and randomly modifying HF at the beginning of each resuspension cycle within a ±10% band. (The grey values correspond to the viable cell concentration (●) and viability (■) obtained with no control action.)
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References


Appendix A
Printouts of Programs

A printout of the code for the model-based observer (MBO) control algorithm developed in this work will be presented in Appendix A.1; this example code refers to the manipulation of $R_{\text{Anti}}$ in repeated batch operation. The code encompasses a main program where the overall control strategy is laid out, merging the algorithms of the process controller and the model adaptor, and calling several sub-routines, as necessary (shown in bold in the code). Fortran 77 was used throughout; the results obtained when testing this algorithm were presented and discussed in Chapter 7. In the example shown in this appendix, a sampling frequency of 6 min was used and no sampling noise or hidden mismatches were considered. It should be noted that, when testing the model adaptor algorithm (Chapters 4 and 5), the part of the code referring to the process controller was not considered; similarly, when testing the process controller algorithm (Chapter 6), the subroutine relative to the model adaptor action was not called.

A printout of the code for the specific model employed in the MBO control strategy will be presented in Appendix A.2; this model was briefly introduced in Section 3.4.2. The code was written in Fortran 77 and the example shown here refers to the copy used to simulate the process.

A.1 The MBO Control Algorithm

```
MAIN PROGRAM
REAL*8 C, DEAD, DEADM, E, KC, OUTIN, PM, PMI, PMO, PP, SE, TI, VIAB, VIABM, X,
<AMMO(O: 1500), AMMOM(O: 1500), ANTI(O: 1500), ANTIM(O: 1500), GLUT(O: 1500),
<GLUTM(O: 1500), NT(O: 1500), NTM(O: 1500), OUTM(O: 1500), OUTP(O: 1500),
<RATIO(O: 1500), SP(O: 1500)
REAL*4 COUNTER, DCT, DCT0, DCTL, HF, SF, TIME, TIMECYCLE
INTEGER CYCLE, DT, NTCYCLE, T, T0, T0M, TC, TF, TG, TGM, TMIN, TRUN, TUL, TUL0
```
Appendix A

C DEFINITION OF VARIABLES
C
C AMMO(T) - ammonia concentration in the medium at step T for the process (mg ml⁻¹)
C AMMOM(T) - ammonia concentration in the medium at step T for the model (mg ml⁻¹)
C ANTI(T) - antibody concentration in the medium at step T for the process (mg ml⁻¹)
C ANTIM(T) - antibody concentration in the medium at step T for the model (mg ml⁻¹)
C CYCLE - resuspension cycle number
C DCT - dilution cycle time (h)
C DCTO - dilution cycle time at the beginning of each resuspension cycle (h)
C DCTL - dilution cycle time calculated at previous integration step (h)
C DEAD - concentration of dead cells for the process (cell ml⁻¹)
C DEADM - concentration of dead cells for the model (cell ml⁻¹)
C DT - integration step length (min)
C E - set point error (units as appropriate)
C GLUT(T) - glutamine concentration in the medium at step T for the process (mg ml⁻¹)
C GLUTM(T) - glutamine concentration in the medium at step T for the model (mg ml⁻¹)
C HF - harvest fraction (%)
C KC - proportional gain of the process controller (units as appropriate)
C OUTIN - process output at the beginning of each resuspension cycle (units as appropriate)
C OUTM(T) - model output at step T (units as appropriate)
C OUTP(T) - process output at step T (units as appropriate)
C PM - current model parameter value (units as appropriate)
C PMI - bias value for the model parameter (units of the model parameter)
C PMO - previous calculated model parameter value (units as appropriate)
C PP - model parameter value used to simulate the process (units as appropriate)
C NTCYCLE - total number of resuspension cycles
C NT(T) - total number of cells at step T for the process (cell ml⁻¹)
C NTM(T) - total number of cells at step T for the model (cell ml⁻¹)
C RATIO(T) - ratio between the number of cells in GIb and in D, at step T
C SF - sampling frequency (min)
C SP(T) - set point (units as appropriate)
C T - integration step number within a particular resuspension cycle
C TF - total number of integration steps per resuspension cycle
C TG - step number when glutamine is exhausted for the process
C TGM - step number when glutamine is exhausted for the model
C TI - integral action time of the process controller (units as appropriate)
C TIME - accumulated simulated time within a resuspension cycle (h)
C TIMECYCLE - total accumulated simulated time (h)
C TMN - minimum number of integration steps per resuspension cycle
C TRUN - total simulated time (min)
C TUL - integration step number at resuspension
C TULO - initially set integration step number at resuspension
C VIAB - cell viability for the process (%)
C VIABM - cell viability for the model (%)
C
C Common blocks necessary for transfer of data between all subroutines used.

COMMON /CYCLE/ CYCLE,DCT,DCTO,HF,TF,TC
COMMON /LIMITS/ TUL,TULO,TMIN
COMMON /TIMEPROC/ T0,TG
COMMON /TIMEMOD/ T0M,TGM
COMMON /MOD/ DEADM,NTM,VIABM
COMMON /PROC/ DEAD,NT,VIAB
COMMON /MEDMOD/ GLUTM,AMMOM,ANTI

A2
Appendix A

COMMON /MEDPROC/ GLUT,AMMO,ANTI
COMMON /COUNT/ TIME,TIMECYCLE,OUTIN
COMMON /M_ADAP/ PMI,PMO

C Outputs files for the RATIO(T) values.

OPEN(11,FILE='C:SF6RAT1.OUT')
OPEN(12,FILE='C:SF6RAT2.OUT')
OPEN(13,FILE='C:SF6RAT3.OUT')
OPEN(14,FILE='C:SF6RAT4.OUT')

C Outputs files for the parameter error.

OPEN(15,FILE='C:SF6MAD1.OUT')
OPEN(16,FILE='C:SF6MAD2.OUT')
OPEN(17,FILE='C:SF6MAD3.OUT')
OPEN(18,FILE='C:SF6MAD4.OUT')

C Outputs files for the PROCESS - set point error, dilution cycle time, viable cell concentration, viability and medium component concentrations.

OPEN(19,FILE='C:SF6PROE.OUT')
OPEN(20,FILE='C:SF6PROC.OUT')
OPEN(21,FILE='C:SF6PROM.OUT')

C Outputs files for the MODEL - dilution cycle time, viable cell concentration, viability and medium component concentrations.

OPEN(22,FILE='C:SF6MODC.OUT')
OPEN(23,FILE='C:SF6MODM.OUT')

C Outputs files for the calculation of the cell age distribution.

OPEN(24,FILE='C:SF6PERC1.OUT')
OPEN(25,FILE='C:SF6PERC2.OUT')

C Operational conditions.

DCT=50.
HF=0.80
NTCYCLE=99

TRUN=DCT*60
DT=6
SF=6.
TF=TRUN/DT
TG=TF
TGM=TF
Appendix A

C PROCESS CONTROLLER...
C Process controller parameters.

KC=30
C TI=1000.
SE=0.
DCT0=DCT
TIME=0.
TIMECYCLE=0.
OUTIN=0.

C MODEL ADAPTOR...
C Initial model parameter and mismatch values.

PP=1.798D-10
C PP=2.573D-10 RGLUT
PM=0.90*PP
PMI=PM
PMO=PMI

DO 200 CYCLE=0,NTCYCLE

C Initialisation of simulation parameters, for each resuspension cycle. DCT is always re-set to its
C 'original' value at the beginning of each cycle.

DCT=50.
DCT0=DCT
TF=DCT*60/DT
TUL0=TF
TUL=TUL0
TMIN=0
COUNTER=0.

DO 100 T=0,1000
TIME=T*DT/60.

CALL P_PROC(T)
CALL P_MOD(T,PM,RATIO)

OUTP(T)=ANTI(T)
OUTM(T)=ANTIM(T)

C THE PROCESS CONTROLLER ALGORITHM...

C Writing the value of RATIO(T) to the output files...

IF (CYCLE.LE.24) THEN
WRITE(11,*) CYCLE,TIMECYCLE+TIME,RATIO(T)
ELSE
  IF (CYCLE.LE.49) THEN
    WRITE(12,*) CYCLE, TIMECYCLE+TIME, RATIO(T)
  ELSE
    IF (CYCLE.LE.74) THEN
      WRITE(13,*) CYCLE, TIMECYCLE+TIME, RATIO(T)
    ELSE
      WRITE(14,*) CYCLE, TIMECYCLE+TIME, RATIO(T)
    ENDIF
  ENDIF
ENDIF

DCTL=DCT

C It is assumed that DCT has to be at least 25% of its ‘original’ value to allow for control action within every resuspension cycle.

TMIN=0.25*DCT*60/DT
IF (T.LT.TMIN) THEN
  GOTO 25
ENDIF

IF (CYCLE.NE.0.AND.T.NE.0) THEN
  C If, based on results obtained in previous integration steps, resuspension will occur prior to TULO, then there is no need to further manipulate DCT; skip all control action!

  IF (TUL.NE.TULO) THEN
    DCT=DCTL
    GOTO 50
  ENDIF

C Manipulation of DCT with respect to the information inferred by the model...
C (only applied from the second resuspension cycle onwards and never at the beginning of a cycle)
C
C ******************************************

IF (RATIO(T).LT.0.OR.RATIO(T).GT.6) THEN
  IF (RATIO(T-1).GT.RATIO(T)) THEN
    C If RATIO(T) is outside its pre-defined band and its value has decreased since step (T-1), then re-suspend at the next integer DCT value and do not manipulate DCT with respect to the set point error.
    X=T/10.
    IF (X.NE.INT(X)) THEN
      TUL=(INT(X)+1)*10
      DCT=TUL*DT/60.
    ELSE
      DCT=T*DT/60.
    ENDIF
    GOTO 50
  ENDIF
ENDIF
ELSE
  IF (RATIO(T-1).GT.6) THEN
    A5
  ELSE
    A5
  ENDIF
ENDIF
If \( RATIO(T) \) is within its pre-defined band, but \( RATIO(T-1) \) is not, then re-suspend at the
next integer DCT value and do not manipulate DCT with respect to the set point error. This
situation would have been due to a sudden decrease in the value of \( RATIO(T) \) corresponding
to cells dying due to prior glutamine exhaustion.

\[
X = T/10. \\
\text{IF} (X \neq \text{INT}(X)) \text{ THEN} \\
\quad TUL = (\text{INT}(X) + 1) \times 10 \\
\quad DCT = \frac{TUL \times DT}{60}.
\]

\[
\text{ELSE} \\
\quad DCT = \frac{T \times DT}{60}.
\]

ENDIF
GOTO 50
ENDIF

Manipulation of DCT with respect to the set point error...

DCT is only manipulated with respect to the set point error when the outputs are sampled,
whereas the manipulation according to the cell age distribution inferred on-line by the model
may be enforced in between samplings.

IF (\( T \neq \text{COUNTER} \)) GOTO 50

Calculation of the set point value at the current integration step and of the set point error; these
values may be determined in terms of either antibody or viable cell concentrations.

IF (\( \text{OUTP}(T) \leq 0 \)) \text{ THEN} \\
\quad E = 0.
ELSE \\
\quad \text{IF} (T = 0) \text{ THEN} \\
\quad \quad \text{OUTIN} = \text{OUTP}(T)
\quad \text{ENDIF} \\
\quad SP(T) = 5D-05 \times T + \text{OUTIN} \\
\quad \text{SP}(T) = 500 \times T + \text{OUTIN}
\]

E = \( \text{SP}(T) - \text{OUTP}(T) \)

ENDIF

P Control
\[ C = KC \times E \]

PI Control
\[ C = SE + E \\
\quad C = KC \times (E + 1/TI \times SE \times DT) \]

Calculation of the new DCT value with respect to the set point error; only integer values for
DCT are considered.
Appendix A

DCT = DCTO + INT(C)
IF (DCT * 10.LT.T) THEN
   DCT = DCTL
ENDIF

TF = DCT * 60 / DT

IF (T.EQ.COUNTER) THEN
   COUNTER = COUNTER + SF / 6.
ENDIF

Transfer to the Model Adaptor...

CALL MA(CYCLE, T, ANTI, ANTIM, PM)

C Writing the value of the parameter error to the output files...

IF (CYCLE .LE. 24) THEN
   WRITE(15, *) CYCLE, TIMECYCLE + TIME, (PM - PP) / PP * 100.
ELSE IF (CYCLE .LE. 49) THEN
   WRITE(16, *) CYCLE, TIMECYCLE + TIME, (PM - PP) / PP * 100.
ELSE IF (CYCLE .LE. 74) THEN
   WRITE(17, *) CYCLE, TIMECYCLE + TIME, (PM - PP) / PP * 100.
ELSE
   WRITE(18, *) CYCLE, TIMECYCLE + TIME, (PM - PP) / PP * 100.
ENDIF
ENDIF

C ... the PROCESS...

   NT(CYCLE+1) = NT(T)
   GLUT(CYCLE+1) = GLUT(T)
   AMMO(CYCLE+1) = AMMO(T)
   ANTI(CYCLE+1) = ANTI(T)
   VIAB = NT(T) / (DEAD + NT(T)) * 100.

C ... and the MODEL.

   NTM(CYCLE+1) = NTM(T)
   GLUTM(CYCLE+1) = GLUTM(T)
   AMMOM(CYCLE+1) = AMMOM(T)
   ANTIM(CYCLE+1) = ANTIM(T)
   VIABM = NTM(T) / (DEADM + NTM(T)) * 100.

WRITE(19, *) CYCLE, TIMECYCLE + TIME, E
WRITE(20, *) CYCLE, TIMECYCLE + TIME, DCT, NT(T), VIAB
WRITE(21, *) TIMECYCLE + TIME, GLUT(T) * 1E+03, AMMO(T) * 1E+03, ANTI(T) * 1E+03
Appendix A

WRITE(22,*), CYCLE, TIMECYCLE + TIME, DCT, NTM(T), VIABM
WRITE(23,*), TIMECYCLE + TIME, GLUTM(T) * 1E+03, AMMOM(T) * 1E+03,
<ANTIM(T) * 1E+03

TC = T
TIMECYCLE = TIMECYCLE + TIME

C If this is the end of the last resuspension cycle considered, then stop the simulation,
C otherwise, proceed to the next cycle.

IF (CYCLE .NE. NTCYCLE) THEN
  GOTO 200
ELSE
  STOP
ENDIF

100 CONTINUE

200 CONTINUE

CLOSE(25)
CLOSE(24)
CLOSE(23)
CLOSE(22)
CLOSE(21)
CLOSE(20)
CLOSE(19)
CLOSE(18)
CLOSE(17)
CLOSE(16)
CLOSE(15)
CLOSE(14)
CLOSE(13)
CLOSE(12)
CLOSE(11)

END

SUBROUTINE MA(CYCLE, T, OUTP, OUTM, PM)
REAL*8 C, E, KC, PM, PMO, PMI, SE, TI, OUTM(0:1500), OUTP(0:1500)
INTEGER CYCLE, DT, T

C **********************************************************************************************************
C DEFINITION OF VARIABLES
C
C CYCLE - resuspension cycle number
C DT - integration step length (min)

A8
Appendix A

CE- model error (units as appropriate)
C KC - proportional gain of the model adaptor (units as appropriate)
C OUTM(T) - model output at step T (units as appropriate)
C OUTP(T) - process output at step T (units as appropriate)
C PM - current model parameter value (units as appropriate)
C PMI - bias value for the model parameter (units of the model parameter)
C PMO - previous calculated model parameter value (units as appropriate)
C T - integration step number
C TI - integral action time of the model adaptor (units as appropriate)
C

COMMON /M_ADAP/ PMI,PMO

THE MODEL ADAPTOR ALGORITHM...

DT=6

Model adaptor parameters.

KC=5D-15
TI=1000.

Calculation of the model error.

IF (OUTP(T).LE.0) THEN
  E=0.
ELSE
  E=OUTP(T)-OUTM(T)
ENDIF

P CONTROL

C=KC*E

PI CONTROL

SE=SE+E
C=KC*(E+1/TI*SE*DT)

PMO=PM

Bump-free operation (re-set of bias)...=

IF (CYCLE.NE.0.AND.T.EQ.0) THEN
  PMI=PMO-C
ENDIF

Calculation of the new model parameter value.

PM=PMI+C

RETURN
END
A.2 The Specific Model

SUBROUTINE P_PROC(T)
REAL*8 AMMOIN,DEAD,GLUTIN,OUTIN,RANTI,RGLUT,RHC,SMAX,TD,TR1,VIAB,
<AMMO(0:1500),ANTI(0:1500),CUM(0:150000),GLUT(0:1500),NO(200,0:1500),
<N1(200,0:1500),N2(100,0:1500),N3(50,0:1500),N4(50,0:1500),N5(50,0:1500),
<N6(900,0:1500),NT(0:150000),OLD(0:150000),PERC(0:6),SUM(0:6),TR(1500)
REAL*4 DCT,DCT0,HF,TIME,TIMECYCLE
INTEGER CYCLE,DT,T0,TC,TF,TG,ELEM(0:6),TP(0:6)
C
C DEFINITION OF VARIABLES
C
C AMMO(T) - ammonia concentration in the medium at step T (mg ml⁻¹)
C AMMOIN - initial ammonia concentration in the medium (mg ml⁻¹)
C ANTI(T) - antibody concentration in the medium at step T (mg ml⁻¹)
C CUM(TO) - cumulative glutamine content of cells in GIₐ, which have entered this phase
C at step T₀ (mg cell⁻¹)
C CYCLE - resuspension cycle number
C DCT - dilution cycle time (h)
C DEAD - concentration of dead cells (cell ml⁻¹)
C DT - integration step length (min)
C ELEM(J) - number of age elements in phase J
C GLUT(T) - glutamine concentration in the medium at step T (mg ml⁻¹)
C GLUTIN - initial glutamine concentration in the medium (mg ml⁻¹)
C HF - harvest fraction (%)
C NO(K, T) - number of cells in element K, in GIₐ, at step T (cell ml⁻¹)
C NI (K, T) - number of cells in element K, in GIₐ, at step T (cell ml⁻¹)
C N2(K, T) - number of cells in element K, in S, at step T (cell ml⁻¹)
C N3(K, T) - number of cells in element K, in G₂, at step T (cell ml⁻¹)
C N4(K, T) - number of cells in element K, in M, at step T (cell ml⁻¹)
C N5(K, T) - number of cells in element K, in GIₐ, at step T (cell ml⁻¹)
C N6(K, T) - number of cells in element K, in D, at step T (cell ml⁻¹)
C NT(T) - total number of cells at step T (cell ml⁻¹)
C PERC(J) - percentage of cells in phase J (%)
C RANTI - specific antibody production rate (mg min⁻¹ cell⁻¹)
C RGLUT - specific glutamine uptake rate (mg min⁻¹ cell⁻¹)
C RHC - rate of glutamine chemical hydrolysis (min⁻¹)
C SMAX - maximum amount of glutamine a cell can consume during the GIₐ phase, before
C it has to proceed to the S phase (mg cell⁻¹)
C SUM(J) - number of cells in the J phase, at step T (cell ml⁻¹)
C T - integration step number
C T0 - step number when a group of cells enters GIₐ
C TD - fraction of cells in the D phase which die
C TG - step number when glutamine is exhausted
C TP(J) - duration of phase J (min)
C TR1 - total fraction of cells transferred from GIₐ to S
C TR(K) - fraction of cells in element K transferred from GIₐ to S
C VIAB - cell viability (%)
C
C ******************************************************************************

COMMON /PROC/ DEAD,NT,VIAB
COMMON /PARPROC/ N0,N1,N2,N3,N4,N5,N6,CUM,OLD
COMMON /SUMPROC/ SUM
COMMON /TIMEPROC/ T0, TG
COMMON /CYCLE/ CYCLE, DCT, DCT0, HF, TF, TC
COMMON /INPROC/ GLUTIN, AMMOIN
COMMON /MEDPROC/ GLUT, AMMO, ANTI
COMMON /TRANSITPROC/ TD, TR
COMMON /COUNT/ TIME, TIMECYCLE, OUTIN

C Simulation parameters.

TP(0)=840
TP(1)=600
TP(2)=300
TP(3)=120
TP(4)=120
TP(5)=150
TP(6)=3000

DT=6

DO 2 J=0,6
2 ELEM(J)=TP(J)/DT

RHC=1D-04

SMAX=2.588D-07
RGLUT=2.573D-10
RANTI=1.798D-10

C *************************************************************************

IF (T.EQ.0.AND.CYCLE.EQ.0) THEN

C Initial conditions and cell age distribution.

NT(0)=.09D+06
DEAD=0.
VIAB=100.
GLUT(0)=1D-01
AMMO(0)=1.660D-03
ANTI(0)=0.

GLUTIN=GLUT(0)
AMMOIN=AMMO(0)

WRITE(20, *) CYCLE, TIMECYCLE+TIME, DCT, NT(T), VIAB
WRITE(21, *) TIMECYCLE+TIME, GLUT(T)*1E+03, AMMO(T)*1E+03, ANTI(T)*1E+03

DO 4 K=1, ELEM(1)
4 N1(K,0)=0.
SUM(1)=0.

DO 6 K=1, ELEM(2)
6 N2(K,0)=0.
SUM(2)=0.

DO 8 K=1, ELEM(3)
8 N3(K,0)=0.
SUM(3)=0.
DO 10 K=1,ELEM(4)
10 N4(K,0)=0.
SUM(4)=0.

DO 12 K=1,ELEM(5)
12 N5(K,0)=0.
SUM(5)=0.

DO 14 K=1,ELEM(6)
14 N6(K,0)=0.
SUM(6)=0.

DO 16 K=1,ELEM(0)
16 N0(K,0)=NT(0)/ELEM(0)
SUM(0)=NT(0)

ELSE

IF (T.EQ.0.AND.CYCLE.NE.0) THEN

C UPDATE CONCENTRATIONS ACCORDING TO HF

C Initial conditions and cell age distribution for all, but the first, cycle, considering the previous
C resuspension cycle had TC integration steps.

TF=TC

NT(0)=(1-HF)*NT(CYCLE)
DEAD=(1-HF)*DEAD
VIAB=NT(T)/(DEAD+NT(T))*100.
GLUT(0)=(1-HF)*GLUT(CYCLE)+HF*GLUTIN
AMMO(0)=(1-HF)*AMMO(CYCLE)+HF*AMMOIN
ANTI(0)=(1-HF)*ANTI(CYCLE)
WRITE(19,*) CYCLE, TIMECYCLE+TIME,E
WRITE(20,* ) CYCLE, TIMECYCLE+TIME,DCT, NT(T), VIAB
WRITE(21,* ) TIMECYCLE+TIME,GLUT(T)*1E+03,AMMO(T)*1E+03,ANTI(T)*1E+03

DO 110 K=1,ELEM(0)
110 N0(K,0)=N0(K,TF)*(1-HF)
SUM(0)=SUM(0)*(1-HF)

DO 120 K=1,ELEM(1)
120 N1(K,0)=N1(K,TF)*(1-HF)
SUM(1)=SUM(1)*(1-HF)
DO 130 K=1,ELEM(2)
130 N2(K,0)=N2(K,TF)*(1-HF)
SUM(2)=SUM(2)*(1-HF)

DO 140 K=1,ELEM(3)
140 N3(K,0)=N3(K,TF)*(1-HF)
SUM(3)=SUM(3)*(1-HF)

DO 150 K=1,ELEM(4)
150 N4(K,0)=N4(K,TF)*(1-HF)
SUM(4)=SUM(4)*(1-HF)
Appendix A

C Re-set the number of integration steps for this resuspension cycle to the value equivalent to the
C 'original' DCT value...

TF=DCT*60/DT

ELSE

C Glutamine is consumed by cells in phases G1a and G1b and by chemical hydrolysis.
C Ammonia is excreted to the medium in phases G1a and G1b.
C Antibody is produced and excreted to the medium in phases G1b and S.

GLUT(T)=GLUT(T-1)*(1-RHC*DT)-DT*RGLUT*(SUM(I)+SUM(5))
IF (GLUT(T).LT.0.) THEN
GLUT(T)=0.
ENDIF

AMMO(T)=AMMO(T-1)+(0.1*DT*RGLUT*(SUM(I)+SUM(5)))
ANTI(T)=ANTI(T-1)+DT*RANTI*(SUM(I)+SUM(2))
IF (GLUT(T).EQ.0.) THEN
TG=T
ENDIF

C G1' PHASE
C

C Cells initially evenly distributed through G1' gradually proceed to G1b. No cells from
C the cell cycle enter this phase.

SUM(0)=0.
N0(1,T)=0.
DO 17 K=2,ELEM(0)
   N0(K,T)=N0(K-1,T-1)
   SUM(0)=SUM(0)+N0(K,T)
17 CONTINUE

C G1b PHASE
C

C Cells in the last element of G1' proceed to this phase; cells from the last element of G1a,
C also. In G1b, cells might not flow through the whole phase. Cells can immediately proceed
C to S via a random transition, its probability depending on the cells glutamine content.
TR1=0.
SUM(1)=0.

IF (T.GE.TG) THEN

DO 19 K=1,ELEM(1)
   IF (K.EQ.1) THEN
      NI(K,T)=N5(ELEM(5),T-1)+N0(ELEM(0),T-1)
   ELSE
      NI(K,T)=NI(K-1,T-1)
   ENDIF
   TRI=0.
   SUM(I)=SUM(I)+NI(K,T)

ELSE

DO 20 K=1,ELEM(1)
   IF (K.EQ.1) THEN
      NI(K,T)=N5(ELEM(5),T-1)+N0(ELEM(0),T-1)
   ELSE
      IF (NI(K,T).NE.0.) THEN
         TO=T
         CUM(TO)=0.
      ENDIF
      TRI=0.
      GOTO 22
   ENDIF

C If new cells enter G1b phase, the per cell cumulative amount of glutamine has to be
C reset to zero.

   IF (NI(K,T).NE.0.) THEN
      T0=T
      CUM(T0)=0.
   ENDIF
   TRI=0.
   GOTO 22
ENDIF

C Calculate when cells in each age element have entered the G1b phase...

T0=T-K+1+DCT*CYCLE*60/DT

IF (T0.GE.0) THEN
   IF ((T+DCT*CYCLE*60/DT)-T0.LT.100) THEN
      IF (NI(K-1,T-1).GT.0.) THEN
         C If cells in this age element have been in G1b for less than 10 h (its maximum duration) and if
         C there were cells in the previous element, at the previous integration step, then there might be
         C cells in NI(K,T), as not all cells enter S via the random transition...
         OLD(T0)=CUM(T0)
         CUM(T0)=OLD(T0)+DT*RGLUT
         IF (CUM(T0).LT.SMAX) THEN
            C Fraction of cells in element K which leaves G1b via the random transition.
            TR(K)=-2*DT*RGLUT/(OLD(T0)-SMAX)
         ELSE
            IF (OLD(T0).LT.SMAX.AND.CUM(T0).GT.SMAX) THEN
               TR(K)=1.
            ENDIF
         ENDIF
   ENDIF
   ELSE
      IF (OLD(T0).LT.SMAX.AND.CUM(T0).GT.SMAX) THEN
         TR(K)=1.
      ENDIF
   ENDIF
ELSE
   IF (OLD(T0).GE.SMAX.AND.CUM(T0).GT.SMAX) THEN
      TR(K)=0.
   ENDIF
ENDIF
ENDIF
ENDIF
ENDIF
ENDIF
IF (TR(K).GT.1) THEN
   TR(K)=1.
ENDIF
N1(K,T)=N1(K-1,T-1)*(1-TR(K))
ELSE
   N1(K,T)=0.
   TR(K)=0.
ENDIF
ENDIF
ELSE
   N1(K,T)=0.
   TR(K)=0.
ENDIF
TR1=TR1+TR(K)*N1(K-1,T-1)
22 SUM(I)=SUM(I)+N1(K,T)
20 CONTINUE
ENDIF

C*****************************************************************************
C S PHASE
C*****************************************************************************
C Cells which leave G1b via the random transition enter the first element of S. No transition
C of cells occurs in this phase, just flow throughout the entire phase.

SUM(2)=0.
DO 30 K=1,ELEM(2)
   IF (K.EQ.1) THEN
      N2(K,T)=TR1
   ELSE
      N2(K,T)=N2(K-1,T-1)
   ENDIF
   N2(K,T)=N2(K-1,T-1)
   ENDIF
   SUM(2)=SUM(2)+N2(K,T)
30 CONTINUE

C*****************************************************************************
C G2 PHASE
C*****************************************************************************
Appendix A

C Cells from S proceed to this phase, where cells just flow, once more.

\[
\begin{align*}
\text{SUM}(3) &= 0. \\
\text{DO 40 K} &= 1, \text{ELEM}(3) \\
&\quad \text{IF (K.EQ.1) THEN} \\
&\quad \quad \text{N3(K,T)} &= \text{N2(ELEM(2),T-1)} \\
&\quad \quad \text{ELSE} \\
&\quad \quad \quad \text{N3(K,T)} &= \text{N3(K-1,T-1)} \\
&\quad \quad \quad \text{ENDIF} \\
&\quad \quad \text{SUM(3)} &= \text{SUM(3)} + \text{N3(K,T)} \\
40 &\quad \text{CONTINUE}
\end{align*}
\]

C **************************************************************
C M PHASE
C **************************************************************

C Again, cells flow through the whole phase.

\[
\begin{align*}
\text{SUM}(4) &= 0. \\
\text{DO 50 K} &= 1, \text{ELEM}(4) \\
&\quad \text{IF (K.EQ.1) THEN} \\
&\quad \quad \text{N4(1,T)} &= \text{N3(ELEM(3),T-1)} \\
&\quad \quad \text{ELSE} \\
&\quad \quad \quad \text{N4(K,T)} &= \text{N4(K-1,T-1)} \\
&\quad \quad \quad \text{ENDIF} \\
&\quad \quad \text{SUM(4)} &= \text{SUM(4)} + \text{N4(K,T)} \\
50 &\quad \text{CONTINUE}
\end{align*}
\]

C **************************************************************
C G1a PHASE
C **************************************************************

C In a glutamine-free medium, cells which finish mitosis do not progress to G1a, but to D.
C Otherwise, cells flow through G1a, consuming glutamine.

\[
\begin{align*}
\text{SUM}(5) &= 0. \\
\text{DO 60 K} &= 1, \text{ELEM}(5) \\
&\quad \text{IF (GLUT(T).EQ.0.) THEN} \\
&\quad \quad \text{IF (K.EQ.1) THEN} \\
&\quad \quad \quad \text{N5(K,T)} &= 0. \\
&\quad \quad \quad \text{GOTO 55} \\
&\quad \quad \quad \text{ELSE} \\
&\quad \quad \quad \quad \text{N5(K,T)} &= \text{N5(K-1,T-1)} \\
&\quad \quad \quad \text{ENDIF} \\
&\quad \quad \quad \text{ENDIF} \\
&\quad \quad \text{IF (K.EQ.1) THEN} \\
&\quad \quad \quad \text{N5(K,T)} &= 2*\text{N4(ELEM(4),T-1)} \\
&\quad \quad \quad \text{ELSE} \\
&\quad \quad \quad \quad \text{N5(K,T)} &= \text{N5(K-1,T-1)} \\
&\quad \quad \quad \quad \text{ENDIF}
\end{align*}
\]

C Mitosis has occurred at the end of the M phase; so, the number of cells in the first element
C of G1a is double the number of cells in the last element of the M phase, at step T-1.

N5(K,T) = 2*\text{N4(ELEM(4),T-1)}
ELSE
N5(K,T) = N5(K-1,T-1)
ENDIF
55  \text{SUM(5) = SUM(5) + N5(K, T)}
60  \text{CONTINUE}

\begin{verbatim}
C*******************************************************************************
C D PHASE
C*******************************************************************************

C If there is glutamine available in the medium, then only cells which stay in GIb for its
C maximum duration enter D. After glutamine exhaustion, cells which complete mitosis
C also immediately enter the D phase.

SUM(6) = 0.
IF (GLUT(T).NE.0) THEN
   N6(1, T) = N1(ELEM(1), T - 1)
ELSE
   N6(1, T) = N1(ELEM(1), T - 1) + 2 * N4(ELEM(4), T - 1)
ENDIF
SUM(6) = N6(1, T)
DO 62 K = 2, ELEM(6)
   IF (N6(K - 1, T - 1).NE.0.) THEN
      C Fraction of cells which die.
      TD = 5.56D-03/60. * ((1D+03*AMMO(T))**1.5)
      IF (TD.GT.1/6.) THEN
         N6(K, T) = 0.
         TD = N6(K - 1, T - 1)
      ELSE
         N6(K, T) = N6(K - 1, T - 1) * (1 - DT * TD)
      ENDIF
   ELSE
      TD = 0.
      N6(K, T) = 0.
   ENDIF
   SUM(6) = SUM(6) + N6(K, T)
   DEAD = DEAD + DT * TD * N6(K - 1, T - 1)
62  CONTINUE

C Calculate the total number of dead cells and of viable cells at step T. Also, determine the
C percentage of cells in each phase, i.e., the cell age distribution, at step T and write its value
C to the output files.

DEAD = DEAD + N6(ELEM(6), T)

NT(T) = 0.
DO 70 J = 0, 6
   NT(T) = NT(T) + SUM(J)
70  ENDIF
ENDIF
\end{verbatim}

A17
DO 80 J=0,6
   IF (NT(T).NE.0.) THEN
      PERC(J)=SUM(J)/NT(T)*100.
   ELSE
      PERC(J)=0.
   ENDIF
80 CONTINUE

WRITE(24,*)(TIMECYCLE+TIME,PERC(J),J=0,6)
WRITE(25,*)(PERC(J),J=3,6)

RETURN
END