Optimal control of fed-batch fermentation processes.

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Abstract

Optimisation of a fed-batch fermentation process typically uses the calculus of variations or Pontryagin's maximum principle to determine an optimal feed rate profile. This often results in a singular control problem and an open loop control structure.

The singular feed rate is the optimal feed rate during the singular control period and is used to control the substrate concentration in the fermenter at an optimal level. This approach is supported by biological knowledge that biochemical reaction rates are controlled by the environmental conditions in the fermenter; in this case, the substrate concentration. Since an accurate neural net-based on-line estimation of the substrate concentration has recently become available and is currently employed in industry, we are therefore able to propose a method which makes use of this estimation. The proposed method divides the optimisation problem into two parts. First, an optimal substrate concentration profile which governs the biochemical reactions in the fermentation process is determined. Then a controller is designed to track the obtained optimal profile. Since the proposed method determines the optimal substrate concentration profile, the singular control problem is therefore avoided because the substrate concentration appears nonlinearly in the system equations. Also, the process is then operated in closed loop control of the substrate concentration. The proposed method is then called "closed loop optimal control".

The proposed closed loop optimal control method is then compared with the open loop optimal feed rate profile method. The comparison simulations from both primary and secondary metabolite production processes show that both methods give similar performance in a case of perfect model while the closed loop optimal control provides better performance than the open loop method in a case of plant/model mismatch. The better performance of the closed loop optimal control is due to an ability to compensate for the modelling errors using feedback.

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Author's Declaration

The work presented in this thesis has not been submitted, either in whole or in part for a degree at this or any other university.

Wirat Vanichsriratana

In memory of my father

There are many paths to the top

of the mountain, but the view there

is always the same.

Nomenclature

X is biomass concentration (g/l)

S is substrate concentration (g/l)

P is production concentration (g/l)

μ is specific growth rate (hr^{-1})

μ_{max} is maximum specific growth rate (hr^{-1})

K_S and K_i are rate constants (g/l)

σ is specific substrate consumption rate (hr^{-1})

π is specific product formation rate (hr^{-1})

π_{max} is maximum specific product formation rate (hr^{-1})

$K_{\pi S}$ and $K_{\pi i}$ are rate constants (g/l)

K_x is biomass coefficient constant (-)

K_p is product coefficient constants (g/l)

K_i is substrate inhibition constant (g/l)

Y_{xs} is yield of substrate to biomass (g biomass/g substrate)

Y_{ps} is yield of substrate to product (g product/g substrate)

k_d is decay rate (hr^{-1})

Y_{xs} is yield of biomass from substrate (g biomass/g substrate)

m_s is substrate used to maintenance biomass (g substrate/g biomass $\cdot$ hr^{-1})

α is product formation coefficient in growth phase (-)

β is product formation coefficient in stationary phase (g product/g biomass $\cdot$ hr^{-1})

k_p is product decay rate (hr^{-1})

D is dilution rate which is the ratio of feed rate and reactor working volume (hr^{-1})

V is culture volume (l)

F is substrate feed rate ($l \cdot hr^{-1}$)

S_f is substrate concentration in the feed (g/l)

H is Hamiltonian

λ is costate variable

OLOFP is open loop optimal feed rate profile

CLOC is closed loop optimal control

CVI is control vector iteration

LQ is linear quadratic

TPBVP is two-point boundary-value problem

MPBVP is multi-point boundary-value problem

OUR is oxygen uptake rate

CER is carbon-dioxide evolution rate

DOT is dissolved oxygen tension

RQ is respiration quotient

EVOP is evolution operation

PHB is poly-β-hydroxybutyric acid

OTR is oxygen transfer rate

ODE is ordinary differential equation

Chapter 1. Introduction

1.0 Introduction

The history of fermentation (Scragg, 1988) can be traced back to 6000 BC when brewing was introduced by the Sumerians. The Egyptians used yeast in bread making around 4000 BC. Cheese and yogurt were produced before 1865. However, it was not until 1920 that research gained better understanding of microbial physiology and subsequently in 1940 that industrial scale production of the first antibiotic - penicillin - was attained. Today, more than 12,000 tonnes of penicillin are produced each year together with other antibiotics such as streptomycin, erythromycin, tetracycline, etc. With the advent of genetic engineering, production of several complex compounds such as monoclonal antibodies and human hormones by fermentation processes has become a reality.

Antibiotics are examples of secondary metabolites produced by micro-organisms and they are also the majority of metabolites being produced nowadays. Secondary metabolites are produced through biosynthetic pathways from primary metabolites, which are essential and vital to all living organisms. Examples of primary metabolites are amino acid, polysaccharides, lipids and other cell constituents (Staunton, 1978).

Although secondary metabolites are non-essential to life, they are important to the organisms that produced them. The role played by secondary metabolites is, however, obscure but usually is associated with survival against other micro-organisms (Porter and Fox, 1993).

As the importance of these metabolites, both primary and secondary, become recognised particularly to the pharmaceutical purpose, the fermentation process has been explored as a tool to produce these desired products. In a fermenter, sometimes called 'bioreactor',

microbial, mammalian or plant cells are cultured to produce the desired metabolite. Many methods have been used to increase the productivity in fermentation processes. These include the exploitation of genetic engineering, strain improvement, media development and bioreactor design.

The main function of a bioreactor (Cliffe, 1988) is to provide a favourable environment for the micro-organisms to achieve the optimal growth and/or product formation. Optimisation of fermentation parameters such as pH, temperature, dissolved oxygen, etc. is therefore used in parallel with strain and medium improvement to yield an increased productivity of the fermentation processes.

During the fermentation operation, these parameters especially substrate concentration in the reactor are maintained at the favourable levels or profiles for maximum production until the end of the batch. The determination of these parameter profiles to achieve some objective functions is known in the control literature as the dynamic optimisation or optimal control problem.

The substrate concentration in the fermenter has been emphasised here because of its importance as micro-organisms' main energy source and raw material for metabolism. Moreover, the substrate concentration also has an effect on metabolite production and controls microbial growth and production phase (Rose, 1979). However, the difficulty on on-line measurement of the substrate concentration has deterred the application of this process optimisation. It is not until recently that a reliable on-line estimation of the substrate concentration has been developed and implemented in a large scale production of antibiotic (Zhang, *et al.*, 1996) and hence stimulate the main theme of this thesis for the process optimisation using the optimal substrate concentration profile. This proposed

optimisation approach will be applied to both primary and secondary metabolite production processes.

In the next section, the applications of control theory, particularly optimal control, applied to fermentation processes are reviewed. A brief summary of a model-based control will also be introduced; this control scheme uses process models explicitly as in the optimal control method for determining required control actions. It will also be used later in our proposed optimisation method. The optimisation problems of fed-batch fermentation processes by conventional methods used in the literature can then be described and compared with the proposed method.

1.1 Optimal Control and Application of Control Theory to Fermentation Processes.

In process control, there are three basic control objectives (Stephanopoulos, 1984). These objectives are:

1. Suppressing the influence of external disturbances.

2. Ensuring the operational stability of a process.

3. Optimising the performance of a process.

The application of control theory to fermentation processes also follows these objectives particularly to improve the process performance.

Melin *et al.* (1982) applied direct digital control using an adaptive control algorithm to control media sterilisation, and control temperature and pH of the culture during fermentation. The results showed the robustness of the adaptive control approach although the models were simple linear models derived from an input step response. Bastin *et al.*

(1982) modelled and used the adaptive control technique to control a waste water treatment process. The authors showed that the nonlinear models that represented the process can be simplified to just first order linear models by linearising them around nominal steady state in this case. The stability of adaptive algorithms for estimation and control of fermentation was described by Dochain and Bastin (1985). Four examples together with simulation results were given:

1. on-line estimation of specific growth rate and substrate concentration by measuring biomass concentration.

2. on-line estimation of specific growth rate, substrate and biomass concentration by measuring dissolved oxygen in the fermenter.

3. adaptive regulator control of an unstable anaerobic process.

4. adaptive regulator control of substrate in a fed-batch process.

Williams *et al.* (1984; 1986) applied an adaptive control for on-line control of baker's yeast fermentation. The authors used sequence of pseudo-linear models to describe the fermentation process that was highly nonlinear and time-varying. This approach was applicable to slow systems like the fermentation process. The advantage of this method was that inaccessible state variables could be ignored and the control law was formulated only from measuring state variables. This technique can be further improved when enhanced with a state estimator of those inaccessible. In the paper, substrate feed rate and agitation speed were used to control dissolved oxygen tension (DOT), carbon-dioxide evolution rate (CER), respiration quotient (RQ) and alcohol in the exhaust gas. The control objective was to control DOT above 10 % and RQ at 1.05, conditions well known for yeast production. The results also showed insufficient performance of a Single-Input and Multi-Output (SIMO) structure for this process, which can be achieved by a Multi-Input and

Multi-Output (MIMO) form. To improve the adaptive control technique, Montgomery, et.al (1985) and Williams and Montgomery (1986) also presented the idea of reconstruction of process variables for checking the accuracy of parameter estimation. With this approach, the performance of the adaptive control was improved. The problem of parameter convergence that affected the short time process was also mentioned. In this case, a priori initial values of parameters can be used to reduce the convergence time.

Montague *et al.* (1985) studied the application of parameter adaptive control to the fed-batch penicillin fermentation. Bajpai and tubular reactor models were used in the study. An extended Kalman filter was applied to estimate biomass and substrate concentration by using CER measurement. Biomass was controlled to follow a desired trajectory by manipulation of sugar feed rate. A Generalised Predictive Controller (GPC) was used as an adaptive control algorithm.

The adaptive control was also applied by Samaan *et al.* (1990) to an alcoholic fermentation. Substrate feed rate was used as a control variable to control substrate level in the fermenter. Dochain and Bastin (1990) applied the adaptive control approach to fed-batch fermentation processes which exhibited substrate inhibition behaviour.

Dochain (1990) extended a Single-Input and Single-Output (SISO) linearising adaptive control to a MIMO in bioprocesses. In the paper, singular perturbation was used to reduce model order. An adaptive linearising control law was derived for three processes: yeast growth, anaerobic digestion and activated sludge. Good simulation results from applying the control scheme to regulate dissolved oxygen and pollutant levels in the activated sludge process were obtained which showed the ability of this approach to cope with variation of disturbances and uncertainty due to variation in model parameters and singular perturbation approximation.

Roux *et al.* (1992) used an adaptive Linear Quadratic Gaussian (LQG) approach to control substrate concentration in ethanol production running in continuous mode. A SISO linear time-varying model was used to represent the process and dilution rate was used as a control variable. Good results in both servo and regulation modes were obtained in experimental tests. An adaptive pole placement control (Dahhou, *et al.*, 1991b) and an L/A control (Dahhou, *et al.*, 1992a; Vigie, *et al.*, 1991) were also used on the same process. The L/A control is a nonlinear control approach that takes into account the positive constraints on control and state variables by a logarithmic transformation.

Dahhou, *et al.* (1991a, 1992b) applied an adaptive predictive control technique to a continuous biomass production process. Dilution rate was used as a manipulated variable to control biomass concentration.

Keulers *et al.* (1993; 1994) controlled specific growth rate, ethanol concentration and dissolved oxygen tension (DOT) in a fed-batch bakers' yeast fermentation. The specific growth rate was estimated by an observer, which was designed based on stoichiometry of oxygen uptake rate (OUR) and carbon-dioxide evolution rate (CER) with growth rate. Air flow rate and stirrer speed were used to control the dissolved oxygen tension while glucose feed rate was used to control the specific growth rate and the ethanol concentration.

Many of the control approaches that are used to improve the performance of the fermentation processes can be characterised as servo or regulator techniques in which set-points of these processes are maintained at a constant level or follow desired trajectories. However, the problem remains of what the optimal set-points or profiles are. Determination of these profiles for optimising the processes under certain criteria, thus leads to an area of optimal control.

The objective of optimal control can be stated (Kirk, 1970) as "to determine the control signals that will cause a process to satisfy the physical constraints and at the same time minimise (or maximise) some performance criteria". The problem of optimal control when solved by the calculus of variations method is known as a two-point boundary-value problem (TPBVP). A characteristic of this problem is that initial conditions of state variables and final conditions of costate or adjoint variables are usually known. It is also referred to as a multi-point boundary-value problem (MPBVP) if there are interior points constraints. Several methods are used to solve this problem and they can be divided into direct and indirect methods. Examples for indirect methods are boundary iteration or generalised shooting technique (Noton, 1972; Ramirez, 1989), Quasilinearisation (Kirk, 1970; Noton, 1972; Ramirez, 1989), while examples for direct methods are control parameterisation (Goh and Teo, 1988; Noton, 1972; Teo, *et al.*, 1989; Teo, *et al.*, 1991), Gradient method in function space (Kirk, 1970; Noton, 1972) or sometimes called Control Vector Iteration (CVI), second-variations (Noton, 1972), Conjugate gradients (Noton, 1972), Sequential gradient-restoration (Miele and Wang, 1986; Teo, *et al.*, 1991). There are also other methods that are used to solve the optimal control problem without referring to the costate. Examples of these methods are dynamic programming (Kirk, 1970; Noton, 1972) and Rosen gradient projection (Kirk, 1970; Rosen, 1960; Rosen, 1966).

These methods have the nature of iterative calculation. Optimal control results are therefore obtained generally in open-loop pre-calculated input sequence that would optimise a desired objective function. It is only in very few cases where a closed-loop control problem can be obtained. An example is the Linear Quadratic (LQ) control, in which the objective function is in a quadratic form and a process model is linear. The topic

of linear optimal control is treated extensively in (Anderson and Moore, 1989; Kwakernaak and Sivan, 1972)

Operation of fermentation processes was originally done by operators who controlled the processes following pre-specified trajectories, which had been known from past experience. This method gave a certain satisfaction. However, this can not guarantee optimality. Hence, the optimality that is calculated explicitly based on a kinetic model under chosen criteria is preferable.

The literature on optimising of fermentation processes is substantial, mostly based on the Calculus of Variations, Pontryagin's Maximum Principle. Green's Theorem has also been used for this purpose (Ohno, *et al.*, 1976). The optimal substrate profile was derived by Guthke and Knorre (1981) using the maximum principle. The obtained optimal profile for a wide range of model parameters consisted of sequence of maximum substrate concentration, abrupt or steep descent change to minimum substrate concentration and minimum substrate concentration. The authors showed that the abruptly changing or fast falling of the substrate concentration can be approximated by a batch growth phase that resulted in an sub-optimal substrate concentration profile.

Substrate feed rate is an input of a fed-batch fermentation process. The optimal control problem is therefore to calculate the optimal feed rate in order to optimise the process. The optimal feed rate can be determined using the calculus of variations. The Pontryagin's Maximum principle is also applied due to physical constraints on feed rate. The optimal feed rate has been applied to several processes, such as production of biomass (Cazzador, 1988; Lim, *et al.*, 1986; Weigand, *et al.*, 1979), antibiotic (Lim, *et al.*, 1986), amino acid (Modak and Lim, 1987; Ohno, *et al.*, 1978), alcohol (Hong, 1986; Modak and Lim, 1987) and glutathione (Shimizu, *et al.*, 1991). The general characteristics of optimal feeding

profile for various fed-batch fermentation were described by Modak *et al.* (1986) and the computational algorithms were implemented by Lim, *et al.* (1986).

With the feed rate appearing linearly in the system equations, singular control is often inevitable. The methods to solve the singular problem have been, however, limited to be effective only to low order processes due to computational reasons. As a gradient between the Hamiltonian and feed rate is indirectly dependent on the feed rate during the singular period, the computation efficiency becomes a problem and it can take a long time for a solution to converge (Terwiesch, *et al.*, 1994). Moreover, the sufficient condition for optimality in singular control is not satisfied (Noton, 1972). Thus, the singular problem is one that should, if possible, be avoided. Several methods have been used to avoid the singular control problem. Modak and Lim (1989) proposed a method to convert the singular control problem to a non-singular one by introducing another set of state variables instead of the usual ones and used culture volume as the control variable instead of feed rate. This made the control variable (volume) appear nonlinearly in the model and the singular problem changed to a nonsingular one. Kelly's transformation was also used by Hong (1986) to reduce the order of system equations to be handled by Pontryagin's scheme. It was also used to avoid the singular control in the work by Ohno, *et al.* (1978).

Under assumption that a specific fermentation rate can be estimated on-line, Agrawal *et al.* (1989) proposed an algorithm to control fed-batch processes at the optimal specific fermentation rate. They used the simulation result of biomass production as an example. Good control efficiency and maximum productivity were obtained under this algorithm. However, there are still problems of on-line estimation of specific fermentation rate that are difficult to perform if this algorithm were to be adopted. Moreover, in a secondary metabolite production, there might be more than two phases in one batch and therefore

time constraint and other factors, such as fermentation volume, have to be taken into account if maximum product is needed.

Although it has been suggested by Modak *et al.* (1986) that keeping the substrate concentration at a constant level is not necessarily optimal, and a sub-optimal result can be obtained. This was also mentioned by Biryukov (1982). Van Impe *et al.* (1991; 1992) developed the sub-optimal control by controlling substrate concentration constant at levels which maximised bioreaction rates. This needs an iterative search for the switching time from the biomass growth phase to the product formation phase in a secondary metabolite process. Van Impe and Bastin (1993) also extended the work to cover the fermentation processes with multiple substrates. An adaptive control approach was used to control the substrate concentration and specific growth rate (Van Impe, *et al.*, 1992).

Biomass, substrate and product concentration in a fermenter are usually measured by laboratory analysis. However the analysis is usually time consuming, expensive and it may take up to several hours to finish the task. This makes it inconvenient for monitoring and controlling the processes. Therefore many methods have been developed for estimating these state variables on-line. These methods are usually based on state estimation and filtering using linear or nonlinear Kalman filters. Stephanopoulos and San (1984) proposed a method for on-line estimation of specific growth rate. The method was based on elementary balance and used on-line measurement (OUR, CER) as input. Bastin and Dochain (1986) estimated specific growth rate on-line by assuming one known state variable such as biomass, substrate, product or production rate. The method was verified in real-life experiments: a continuous fermentation of lactoserum by *Rhodopseudomonas capsulata*, ethanol production by yeast in a batch process and methane production in an

anaerobic digestion plant. The stability and convergence properties of the algorithm were also described.

Shimizu *et al.* (1989b) proposed an algorithm for on-line estimation of specific growth rate. The algorithm used the macroscopic balance and the extended Kalman filter. The article also discussed the selection of on-line measurements for used in the estimation scheme, and proposed that the condition number of the coefficient matrix should be used as the selection criterion. The extended Kalman filter was also used by Nahlik and Burianec (1988) to estimate biomass concentration in a continuous culture of *Candida utilis* under aerobic condition. The substrate concentration in the fermenter and in the feed stream and dilution rate were used to drive the estimator. Montague *et al.* (1989) derived an adaptive observer technique to estimate biomass. The estimator was based on the previous measurements of biomass, CER and feed rate. Dochain *et al.* (1989) developed an asymptotic observer for on-line state estimation in fermentation processes. The observer was used to estimate biomass and product concentration in the poly-β-hydroxybutyric acid (PHB) production process using measurement of dissolved oxygen and OUR. The results showed a very good fit between the estimation and off-line data analysed from a laboratory. Chattaway and Stephanopoulos (1989) presented an adaptive state estimator for monitoring plasmid instability in a recombinant cell culture. The estimator was a Linear Kalman Filter (LKF) whose parameters were identified simultaneously by a recursive method.

Tsao *et al.* (1991) used an empirical reaction subspace and singular value decomposition for on-line estimation of biomass, sugar and glutamic acid concentration in a fed-batch glutamic acid production process. For this process, the singular value decomposition

showed that only one singular value was significantly larger than others. Therefore, only one on-line measurement was needed and OUR was chosen in this application.

Darouach and Boutayeb (1992) presented a method to estimate states and parameters for a linear time variant SISO singular system. The singular system was first transformed to a canonical observable form, then the parameters were estimated using a recursive least-square method. The state was, consequently, estimated by the Kalman filter in a bootstrap manner. Recently, a reliable on-line estimation of substrate concentration has been successfully developed and implemented in an industrial production plant of oxytetracycline antibiotic (Zhang, *et al.*, 1996).

Many control schemes have been used for controlling the specific growth rate in the fermentation processes. Shioya *et al.* (1985) estimated specific growth rate on-line based on a method proposed by Stephanopoulos (Cooney, *et al.*, 1977; Stephanopoulos and San, 1984) and used a Program controller/Feedback compensator - Model Reference Adaptive Control (PF-MRAC) to control it following the desired trajectory. The method was verified with baker's yeast fed-batch experiments. Takamatsu *et al.* (1985) published their work with simulations of baker's yeast fed-batch fermentation. The optimal feeding was pre-calculated by the Maximum principle. The authors used a computer to control biomass and specific growth rate close to the pre-calculated profile. Four different algorithms based on Program controller/Feedback compensator (PF) were used for controlling the process. The results were also compared. However, there were no real experimental data at this time. Shimizu *et al.* (1989a) continued this work by incorporating Model Reference Adaptive Control (PF-MRAC) into the algorithm to control the specific growth rate of baker's yeast in a fed-batch mode. With introducing dead time in the model and using bang-bang type profile control, quality of baker's yeast was improved as ratio of budding

cells to total cells decreased by 50 % compared with constant control of specific growth rate.

Shimizu *et al.* (1991) used the Maximum principle to calculate a feed profile to maximise glutathione produced by yeast in a fed-batch process. The feed profile was composed of two phases. The first one was used to maintain growth rate at the maximum specific growth rate. The other was used to maintain growth rate at the maximum specific production rate. The model in this process came from mass balance and empirical relations. The specific growth rate was estimated by Kalman filter and the process was controlled by the PF system.

Zeng *et al.* (1992) used model reference adaptive control (MRAC) to control the specific growth rate following a specific profile. Hagander and Holst (1992) used a PI controller to regulate the substrate concentration in the exponential growth phase. Pomerleau and Viel (1992) used adaptive nonlinear control to control the level of ethanol in industrial bakers' yeast production. The substrate feed rate was used as the manipulating variable determined by a nonlinear function of oxygen transfer rate (OTR) and ethanol concentration. One parameter in the control law was time-varying and needed to be estimated on-line.

Process model can be used explicitly not only for process optimisation but also for controller design purpose. In the next section, a control scheme that employs process model explicitly for determining the control law is introduced.

1.2 Model Based Predictive Control

Process models can be used explicitly to design a class of controller called "model based control". Model based predictive control has been the subject of research for many years. Its main concepts were however introduced by Wiener (1942) about 50 years ago. Model

predictive control has been developed independently in many parts of the world and in many names: ANDREA/GERBOIS in France as Model Predictive Heuristic Control (MPHC) (Richalet, *et al.*, 1978), Shell Oil Company in the United State as Dynamic Matrix Control (DMC) (Cutler and Ramaker, 1979), Quadratic Dynamic Matrix Control (QDMC) (Garcia and Morshedi, 1986), IDentification and COMmand (IDCOM) (Froisy and Richalet, 1986), Model Algorithmic Control (MAC) (Mehra, *et al.*, 1982; Rouhani and Mehra, 1982), Internal Model Control (IMC) (Economou and Morari, 1986a; Economou and Morari, 1986b; Garcia and Morari, 1982; Garcia and Morari, 1985a; Garcia and Morari, 1985b; Rivera, *et al.*, 1986), Generalised Predictive Control (GPC) (Clarke, *et al.*, 1987a; Clarke, *et al.*, 1987b), Receding Horizon Tracking control (RHTC) (Kwon and Byun, 1989).

The applications of model predictive control include a furnace (Cutler and Ramaker, 1979), a catalytic cracking unit (Richalet, *et al.*, 1978), a vinyl chloride production plant (Lebourgeois, 1980), a petroleum crude distillation unit (Engrand, 1980), a steam generator (Mehra and Eterno, 1980), a fluidized bed reactor (temperature control) (Lee, *et al.*, 1993), a distillation column (Lee, *et al.*, 1992; Lee and Morari, 1992; McDonald and McAvoy, 1987; Semino, *et al.*, 1993; Zafiriou and Morari, 1988), a rapid thermal processing (Breedijk, *et al.*, 1994). These applications cover a wide range of systems including multivariables, ill-conditioned, time delays and nonminimum phase behaviour plants.

The main strategy of model predictive control is to predict the effect of potential control actions on the future values of the process output over a finite interval and find the best control actions which minimise the objective function, which is usually the sum of squared errors between predicted outputs and desired set-points.

The model predictive control has, therefore, two main parameters that need to be chosen by the designer:

1. Prediction horizon: The process models are used to predict the finite future outputs, which are used to compare with the desired set-points.

2. Control horizon: The sequence of finite future control inputs are determined to optimise the objective function based on the finite future outputs and desired set-points.

However only the first control action is implemented and the optimisation procedure is restarted again for the next finite time horizon. This strategy is called moving or receding horizon, which allow the disturbances and plant/model mismatch to be compensated. With these features, the model predictive control is proved to be popular and practical in industrial process control applications.

IMC control scheme needs an inverse process model that may be difficult to obtain if the process model has time delays and zeros outside the unit circle. Arulalan and Deshpande (1987) developed simplified model predictive control, which did not require an inversion of a process model. The IMC is extended to cover unstable plants by Zafiriou and Morari (1991).

The comparison of IMC and LQG has been considered and shown by Scali *et al.* (1992) for a SISO case. The authors have shown that in the majority of cases, the quality of performance is very similar. However, the design of robustness is more straightforward in IMC, whose robustness could be met directly via IMC filter parameters. The relationship between IMC filter parameters and robustness was shown in (Laughlin, *et al.*, 1986).

A double filter IMC structure was used by Zafiriou and Morari (1988) for controlling ill-conditioned processes. A simpler version was also presented by Semino *et al.* (1993)

Difficulties in process measurements usually arise from long sampling delays, poor signal-to-noise ratio and high cost of measurement devices. These lead to the utilisation of secondary measurements, which can be more frequent, reliable and convenient. A framework for model based inferential control, called Generalised Inferential Control (GIC), was therefore proposed by Lee *et al.* (Lee, *et al.*, 1992; Lee and Morari, 1992) for multi-rate sampled-data systems.

The application of MPC for unstable processes was treated in (Muske and Rawlings, 1993a; Muske and Rawlings, 1993b). The papers also incorporated the exploitation of a state estimator for systems with unmeasured state variables. The problem of multivariable systems with time delay and right-half-plane zeros was also treated in (Jerome and Ray, 1992)

The stability of model predictive control is usually analysed in the receding horizon framework (Bitmead, *et al.*, 1990; Kwon and Byun, 1989; Mayne and Michalska, 1990; Mosca and Zhang, 1992)

Model predictive control uses a linear model as an approximation for a plant, while the real plant is nonlinear. The robustness of model predictive control to compensate this inaccuracy may be enhanced by adding a filter on the input signal to the controller as in internal model control (IMC) (Garcia and Morari, 1982; Garcia and Morari, 1985a) or using a nonlinear model in the model predictive control scheme. Adding a filter to improve robustness was used in (Arulalan and Deshpande, 1987; Yeo and Williams, 1987). McDonald and McAvoy (1987) used a linear model with gain and time constant scheduling to compensate for the nonlinear behaviour and improved control system performance in a distillation tower.

Yeo and Williams (1987) used a bilinear model predictive control for a wider range of accurate representation of the plant than a linear model and also included a filter for correcting the dynamic effects of model inaccuracies.

QDMC extended by using a nonlinear process model was considered by Gattu and Zafiriou (1992). In this version of QDMC, the linear model was obtained by linearisation of the nonlinear model at each sampling time.

Sistu and Bequette (1991) applied nonlinear model predictive control to control temperature in a Continuous Stirred Tank Reactor (CSTR). They included the estimation of model parameters with initial conditions of state variables to cope with plant/model mismatch. The authors handled the constraints on dynamic model by transforming the ordinary differential equations (ODE) into algebraic equations using orthogonal collocation on finite elements as an inner loop. Then they used an optimisation code as an outer loop. Biegler (1991) reviewed several optimisation approaches based on nonlinear programming applied to nonlinear model predictive control. Ali and Zafiriou (1993) included an extended Kalman filter for state estimation in nonlinear model predictive control. A formalisation of parameter tuning was also attempted. This was done by using off-line optimisation to satisfy some specified time-domain criteria such as speed of response and overshoot.

Ozgulsen *et al.* (1993) applied NMPC to a periodically forced continuous stirred-tank reactor (ethylene oxidation process). The authors also demonstrated the use of a nonlinear input-output model incorporated into NMPC instead of a first principles model.

The method used in nonlinear model predictive control usually starts with transforming the process model in an ordinary differential equation (ODE) form into algebraic equations using orthogonal collocation on finite elements so that it can be handled explicitly with

other constraints. The nonlinear programming problem is then solved usually by using a Successive Quadratic Programming method (SQP) (Cuthrell and Biegler, 1987; Renfro, *et al.*, 1987). This method was used by Sistu and Bequette (1991), Eaton and Rawlings (1990). There are many variations of this method, which are also encountered extensively in the optimal control literature particularly on trajectory optimisation (Betts, 1989; Betts and Huffman, 1990; Hargraves and Paris, 1987).

1.3 Problem and Objective

It can be seen from the literature review in previous sections that production improvement in early times was based upon process operator experience. Processes were controlled following some pre-specified conditions or values that would enhance the production. Many control schemes have been used to regulate the processes following these approaches. These conditions are, for example, control of biomass for indirectly control of penicillin production (Montague, *et al.*, 1989), control of ethanol for good production of bakers' yeast (Pomerleau and Viel, 1992) and also control of DOT and RQ to achieve good quality of bakers' yeast production (Williams and Montgomery, 1986; Williams, *et al.*, 1984; Williams, *et al.*, 1986) . These situation are actually the cascade control in which key environmental variables that have effect on the fermentation are controlled. It has been mentioned by Lee and Weekman (1976) that the profit from process control arose mainly from the optimisation of the operating conditions, i.e., determination of the optimal set-points, rather than from regulation. A well functioning regulatory layer is necessary, however, to implement the actions dictated by the optimising layer.

A systematic optimisation method such as the calculus of variations and particularly the Maximum principle of Pontryagin has been used extensively in the literature. Most of the research on optimisation that has been concerned with fermentation processes are operated

in a specific mode of operation called fed-batch mode. This is due to the fact that fed-batch fermentation is widely used in industry and has many advantages over other modes especially for secondary metabolite production. Moreover, an ability to manipulate feed rate also forms a challenging problem in control and optimisation of a fermentation process. Details on different modes of fermentation will be given in Chapter 2.

There are two problems arising, however, in applying variational methods to the fermentation process. The first is that a singular control situation may occur during operation since the control input or feed rate appears linearly in the Hamiltonian. The other problem is that the obtained optimal feed rate profile from the variational method is in open loop form and can suffer quite severely when the model parameters are not exactly known in real applications. A closed loop control can also be obtained but in a very rare and specific case such as a Linear Quadratic Regulator (LQR), in which the process model is linear and the cost function is a quadratic formation of state and control variables.

Therefore in this thesis, we propose an approach that avoids the singular control problem and also converts the problem of open loop control into a more tractable closed loop one. In the proposed method, we divide the problem of optimisation of a fermentation process into two parts. Firstly, the optimal substrate concentration profiles that has direct effect on the biochemical reactions in the fermentation process is derived. Then, a controller is designed to track the obtained optimal substrate concentration profile. With this two-step approach, the singular problem is avoided, as the substrate concentration typically appears as a nonlinear function in the Hamiltonian. In order to study an effect of substrate concentration profile to the optimisation of the fed-batch fermentation process, other environmental variables in the fermenter such as temperature, pH, dissolved oxygen are assumed to be maintained at their nominal values.

Since the optimal profile of the substrate concentration has been derived, the substrate feed rate then naturally becomes a control variable to manipulate the substrate concentration. However, it is not until recently that the on-line estimator of substrate concentration becomes available (Zhang, *et al.*, 1996) and makes the proposed method applicable.

It is also the aim of this thesis to show that the optimal feed rate profile presented in the last few years can be put under the proposed framework where the optimisation problem is separated into two parts as:

1. The optimal substrate concentration profile determination.

2. Controller design for which model-based control is used in this work.

The main advantages of the proposed method can be summarised as:

- The proposed method operates under feedback control. The feedback information can therefore be used to improve control performance.

- There is flexibility in designing different types of controllers for controlling the processes since the optimal set-points have been determined.

- This method avoids the singular problem, which occurs when the desired feed rate is determined from the calculus of variations or Pontryagin's maximum principle.

- Most of all, it is transparent for use in industry where the operators prefer to regulate the controlled variables such as the substrate concentration at pre-specified values to adjust feed rate following the pre-determined pattern.

To clearly show the area where the proposed research belongs, a diagram showing related topics on optimal control of fed-batch fermentation processes is shown in Figure 1-1. In the figure, the optimal control problem can be solved directly by iterative search using optimisation methods (such as dynamic programming, gradient projection (Kirk, 1970;

Rosen, 1960; Rosen, 1966) or nonlinear programming) or indirectly by exploiting the optimisation methods to solve the two-point boundary-value problem (TPBVP) (such as boundary iteration, Quasilinearisation, Gradient methods in function space, second-variations or Sequential gradient-restoration). An Optimal feed rate profile is an optimal solution when a constraint optimisation or calculus of variations is applied to a fed-batch fermentation process (Cazzador, 1988; Hong, 1986; Lim, *et al.*, 1986; Modak and Lim, 1987; Ohno, *et al.*, 1978; Shimizu, *et al.*, 1991; Weigand, *et al.*, 1979). However, as the substrate feed rate is linear in the system and Hamiltonian equations, the singular control problem is often unavoidable. Much work has been done to overcome this problem (Hong, 1986; Modak and Lim, 1989; Ohno, *et al.*, 1978).

Optimal specific bioreaction rates such as specific growth rate have also been determined and controlled by many algorithms such as PF and PF-MRAC (Shimizu, *et al.*, 1991; Shimizu, *et al.*, 1989a; Shioya, *et al.*, 1985; Takamatsu, *et al.*, 1985). However the specific reaction rates are rate variables, which can not be measured directly and are subject to error especially if the measurement is noisy.

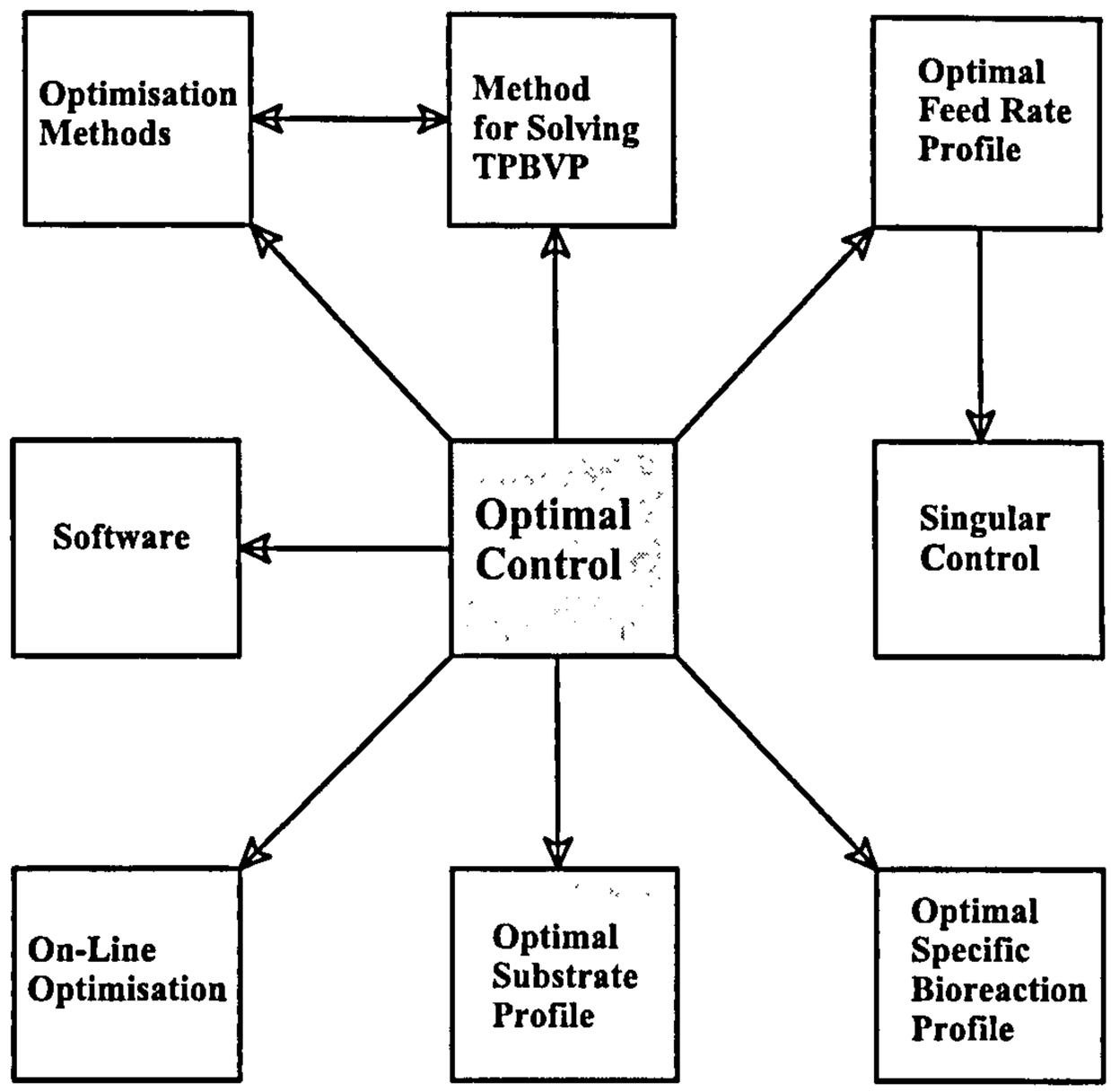


Figure 1-1 Summary of related topic on optimal control of fermentation processes

Since the substrate concentration can be estimated on-line (Zhang, *et al.*, 1996), the optimal substrate concentration profile is determined and controlled by a model predictive control scheme in this thesis (shown in a lighter grey box in Figure 1-1). Noting that the optimal substrate concentration profile is not necessarily constant at those levels which maximise the specific bioreaction rates. Keeping the substrate concentration constant at these levels can lead to sub-optimal control (Biryukov, 1982; Van Impe, *et al.*, 1991; Van Impe, *et al.*, 1992).

On-line optimisation has also been studied in many cases where the process model is not accurate or not available (Chang and Lim, 1989; Chang and Lim, 1990; Chang, *et al.*, 1988; Hamer and Richenberg, 1988; Hilaly, *et al.*, 1994; Rolf and Lim, 1984; Rolf and Lim, 1985; Semones and Lim, 1989). There are also a number of software programs being developed for the optimal control calculation. Although this software has not been particularly designed for using in fermentation processes, it might still be useful. Examples

are ANalysis and DEsign of Controlled Systems (ANDECS) (Mehlhorn, *et al.*, 1994) and Optimal Control CALculator (OCCAL) (Schopf and Deuflhard, 1994). The later has symbolic computational capability, which is particularly useful for an analytical calculation needed for solving the two-point boundary-value problem or deriving the singular feed rate in the optimal feed rate profile determination. Many of these packages solve a constrained optimisation problem by transforming it into a nonlinear programming problem, which can then be solved by a sequential quadratic programming (SQP) method (Bestle and Eberhard, 1994; Fan, *et al.*, 1988; Gill, *et al.*, 1994; Steinbach, 1994).

1.4 Outline of the Thesis

The thesis is structured as follows:

Chapter 1 starts with the presenting of motivation on optimisation of fermentation processes. It is followed by a review of literature in this area. The problem is then pointed out from the literature and the solution, which forms the theme of this thesis is proposed.

Chapter 2 will elucidate the type and characteristic of fermentation processes especially those operated in fed-batch mode. The modelling of fermentation processes is also presented with the aim to help understanding of material in the following chapters.

Chapter 3 will derive the open loop optimal feed rate profile, which is used to optimise fermentation processes in the literature. The results will then apply to two classes of fermentation processes, which are characterised by production behaviour, called primary and secondary metabolite production processes.

In Chapter 4, the proposed method is derived. The method separates an open loop optimal control into two parts. Firstly, the optimal substrate concentration profile is derived and followed by the design of controller, where the predictive control is used in this study.

The proposed method and the open loop optimal control method are then compared in Chapter 5. The relationship, advantages and disadvantages of each method are explored. Examples on primary and secondary metabolite production are used to show the relationship and demonstrate for comparison. Then some conclusions are drawn for the general classes of fermentation processes.

The thesis will be concluded in Chapter 6 and the potential topics for further investigation are suggested. It is followed by a series of appendices.

The thesis is organised as shown in the Figure 1-2

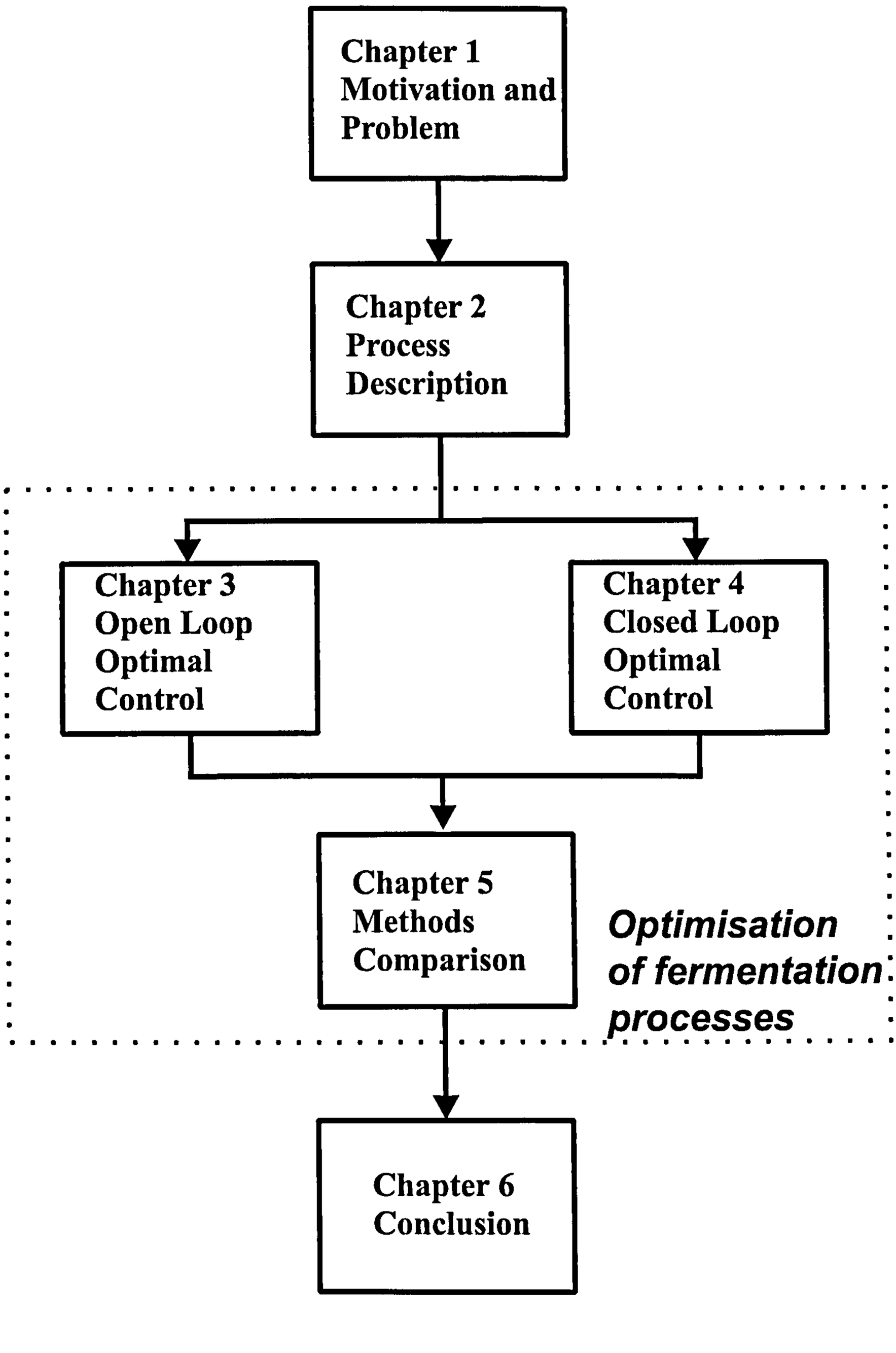


Figure 1-2 Diagram shows outline of the thesis

Chapter 2. Fermentation Process Description, Modelling and Analysis

2.0 Introduction

The term 'fermentation' comes from the Latin verb 'fervere', which means "to boil". It was first used to describe the boiling appearance of carbon dioxide bubbles during the catabolism of sugar in suspended cultures of yeast. However, the term 'fermentation' has been used later by industrial microbiologists to describe processes for microbial production of biochemical compound, through mass culture of micro-organisms (Stanbury and Whitaker, 1993).

2.1 Primary and Secondary Metabolite Production

Fermentation processes can be generally divided into two classes, depending on product formation. These are fermentation for primary or secondary metabolites production.

In the primary metabolite production process, the metabolites are synthesised directly from the primary metabolism. Main functions of primary metabolites are to provide energy and important biochemical compounds necessary for microbial activity and growth. Examples of primary metabolites are proteins, ethanol and other cell constituents. Growth and primary metabolite formation occur almost in parallel. Biomass production is therefore referred to as one of the primary metabolite productions, and conditions that are suitable for primary metabolite production are also suitable for microbial growth.

In the secondary metabolite production process, the metabolite production is associated with limited or sub-optimal growth. It was initially believed that primary metabolism and

secondary metabolites formation occur at separate times and secondary metabolites are synthesised after microbial growth has ceased (Figure 2-1b).

There are generally two phases taking place for the secondary metabolite production: growth phase and production phase. The growth phase is sometimes called the trophophase and the subsequent phase, in which the secondary metabolites are produced is called the idiophase. The enzymes for producing secondary metabolites are synthesised during the trophophase and are used in the idiophase. Therefore, in this type of fermentation, it starts with growth and primary metabolism, and secondary products are formed afterwards. However, the production of secondary metabolites does not necessarily start after the growth has stopped. It is not entirely separate from the growth phase (Figure 2-1c). Many antibiotics and vitamins belong to the secondary metabolite fermentation.

Diagram showed different production phases for primary and secondary metabolite production is shown in Figure 2-1 (Crueger and Crueger, 1989; Wang, *et al.*, 1979)

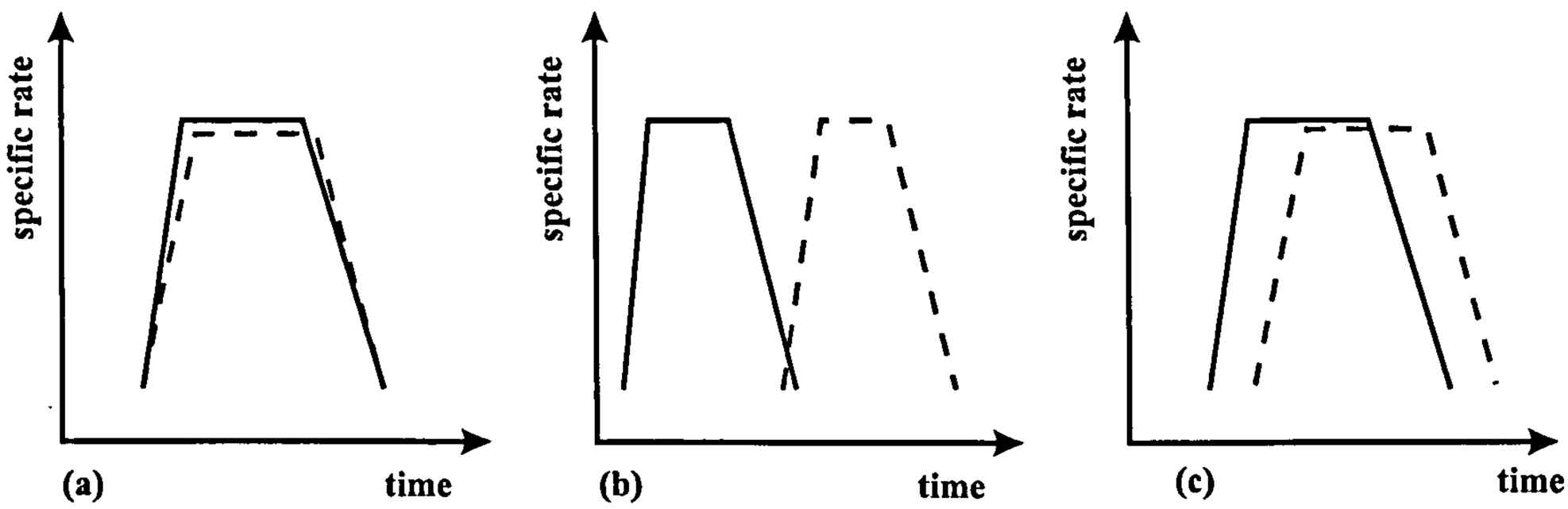


Figure 2-1 Diagram showed different phases for primary (a) and secondary (b-c) metabolite production.

Specific growth rate (——), Specific product formation rate (---)

This classification is quite general and may not cover all the microbial metabolite productions since the separation line between primary and secondary metabolites is

sometimes vague. Moreover, the fermentation characteristics depend not only upon the types of metabolite production but also composition of culture media and regulation of production strains. This classification is, nevertheless, a general assortment of fermentation category. The relationship between the specific growth rate and the specific product formation rate in Figure 2-1 is also referred to as: (a) growth-associated, (b) nongrowth-associated, and (c) mixed-growth associated product.

2.2 Mode of Operation in Fermentation Processes

There are three modes of operation for fermentation processes namely: batch, fed-batch and continuous. The mode of operation is usually decided by types of products being produced. These modes are briefly described in the following sections which will, however, concentrate more on the fed-batch mode because of its importance in production and advantages over the others.

2.2.1 Batch process

A batch process is a closed system. A vessel containing appropriate medium is inoculated by a strain of micro-organisms. The process starts by adjusting environmental conditions (pH, temperature, etc.) suitable for growth of the micro-organisms. Biochemical composition in the fermenter is changed due to metabolism. During the entire fermentation, nothing is added except oxygen in the form of air (in case of aerobic fermentation), antifoam, and acid or alkali for controlling pH. Typically, there are four phases of growth during the entire period of fermentation. These phases are lag, log, stationary and death (Figure 2-2) (Crueger and Crueger, 1989).

Lag phase occurs when the cells are first transferred into the fermenter. There is no growth in this period during which the micro-organisms adapt to the new environment.

Log phase starts after the lag phase. The growth rate is constant during this period. The relationship between biomass and time can be plotted on a semilogarithmic graph and results in a straight line, so the name log phase. After a period of time, the substrates become exhausted and toxic metabolites may form. As a result, growth rate decreases and some of the cells die. When the rate of growth and death are equal, there is no overall increase in biomass. This is called the stationary phase. Eventually, due to lack of essential nutrient and accumulation of toxic metabolites, the cells die (death phase). These phases are shown in Figure 2-2.

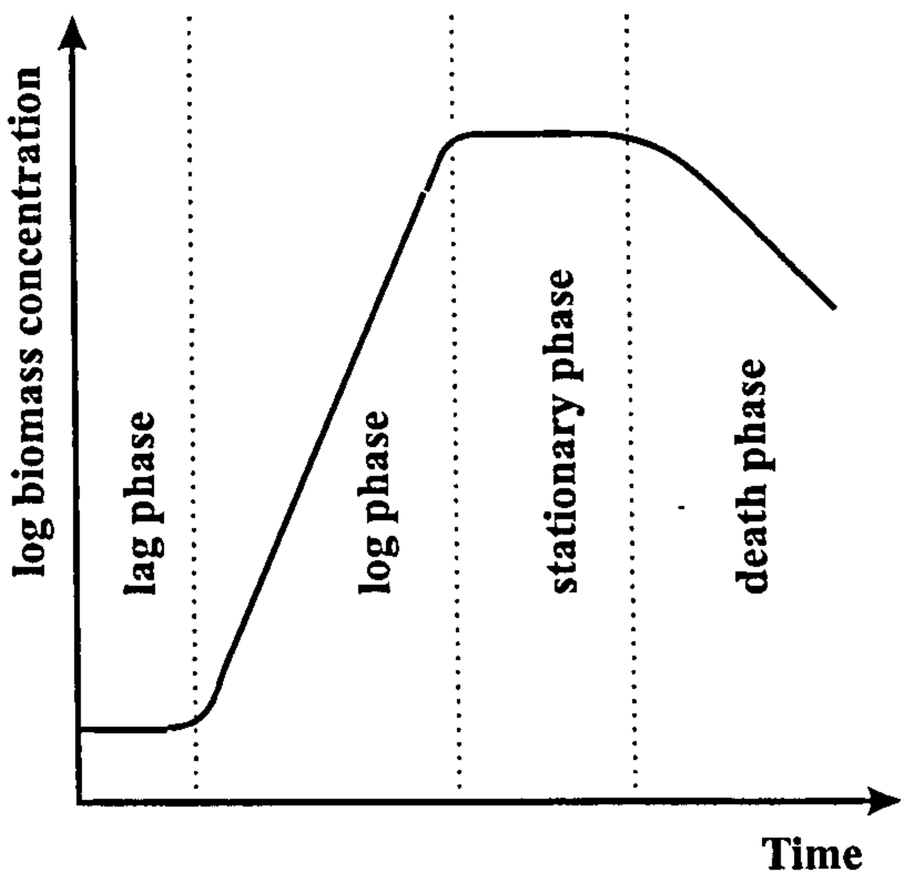


Figure 2-2 Growth phases in a batch fermentation

2.2.2 Continuous process

In batch culture, growth and product formation will cease after some finite time interval. However, microbial growth can be maintained by continually adding substrate into the fermenter and the batch process becomes fed-batch or continuous process. The continuous fermentation process is an open system. Substrate is fed into the fermenter continuously and an equivalent amount of culture in the fermenter is simultaneously withdrawn. By continuously adding substrate, the concentration of the limiting substrate can be

maintained and microbial growth can be prolonged. Moreover, a steady state can be achieved such that the biomass and product concentration, specific growth rate and other culture environment (e.g. substrate concentration) do not change with time. Therefore, continuous culture provides a valuable tool for studying the response of micro-organisms to change in environment conditions such as substrate concentration, pH and temperature etc.

2.2.3 Fed-batch process

The term "fed-batch" was first introduced by Yoshida *et al.* (1973) to describe a technique in microbial processes where one or more nutrients are supplied continuously or sequentially to the fermenter during cultivation and in which the products remain in the containment until the end of the operation. Since substrates are added into the fermenter, the substrate concentrations in the fermenter are maintained at preferable levels, which suit the micro-organisms growth and metabolite production. The fed-batch technique is, in fact, identical to semi-batch technique, which is used extensively in chemical engineering. The term "fed-Batch" is used in microbial processes because the substrates added into the fermenter are nutrients consumed by micro-organisms.

The fed-batch operation is used widely for production of antibiotic in industry since it can overcome drawbacks of secondary metabolite production happening in other modes of operations. For example, it can eliminate catabolite repression in batch process and avoid the genetic instability of mutant production strain in continuous process. Moreover, fed-batch mode also gives the operator the freedom of manipulating the process via substrate feed rate. This provides the challenge of effectively controlling and optimising the fed-batch process. The advantages of using fed-batch mode has been summarised (Parulekar and Lim, 1985; Yamane and Shimizu, 1984) as follows:

1. Reduction of substrate Inhibition: substrate inhibition can be reduced by gradually adding substrate into the fermenter. The substrate concentration is, therefore, kept at low level without causing any inhibition effect.

2. High cell concentration: As high level of substrates or nutrients might cause substrate inhibition, the gradually adding of substrate to the fermenter could reduce this effect and therefore results in the optimal microbial growth and, therefore, high cell concentration.

3. Reduction of the glucose effect: Glucose effect is usually referred to in a yeast production process as a circumstance in which ethanol is produced even in the presence of sufficient dissolved oxygen if an excess of sugar is present in the fermenter. The fed-batch technique can, therefore, be used to improve production by adding sugar when the micro-organism needed but not too much to produce ethanol.

4. Reduction of the catabolite repression: Catabolite repression is a phenomenon in which a micro-organism is provided with a lot of rapidly metabolised carbon-energy source such as glucose. This causes the increase in ATP inside the cells, which leads to the repression of enzyme biosynthesis, and consequently causing a slower metabolism of the energy source. Fed-batch process, therefore, avoids the catabolite repression by gradually feeding of glucose to keep glucose concentration in the fermenter at the low level.

5. Auxotrophic mutants or nutritional mutant. This mutant will grow and produce a lot of biomass under the excess of substrate or nutrient. This results in a very small amount of metabolite product being produced. In contrast, the starvation of nutrient also results in small amount of biomass and hence small amount of product. Therefore, fed-batch process is suitable for cultivation of this mutant culture as the nutrient is fed into the

process at the controlled rate. Therefore, the nutrient level can be kept at the optimal level. This mutant is usually used in industrial amino acid production processes.

6. Extension of operation time: The fermentation time can be extended by adding substrate at the end of a batch operation. If a product is still produced, the final product concentration will also be increased by the extension of time. However, operating cost also increases and has to be considered together with the increase of the product concentration. The extension of operation time is, however, suitable for a nongrowth-associated product formation process, in which microbial production of the desired metabolite at the later stage in the batch (after microbial growth) can be extended. In the fed-batch mode, the substrate was fed to the fermenter to maintain the product formation condition and prolonged the production time.

7. Replacement of water lost by evaporation: In aerobic fermentation, large amount of water may be lost through aeration, the fed-batch technique can be used to replace the water that was lost during the process.

8. Decreasing viscosity of the broth: High viscosity usually causes problems of oxygen transfer in the fermentation process. The fed-batch technique can be used to reduce the viscosity in the process either by feeding substrate or even water to dilute the culture and, hence, increases the oxygen transfer.

A diagram of a basic fermenter is shown in Figure 2-3 with its notation. The diagram is applied for the three modes of operation under the following situations:

- If there is no substrate feed rate and culture removal, it becomes a batch fermentation.

  ($F_{in} = F_{out} = 0$)

- If substrate is fed and culture is removed, it becomes a continuous fermentation and both substrate feed rate and culture removal have to be equal. ($F_{in} = F_{out}$)

- If there is only substrate feed but no culture removal, it becomes a fed-batch fermentation. ($F_{out} = 0$)

These conditions for different modes of fermentation are summarised in Table 2-1.

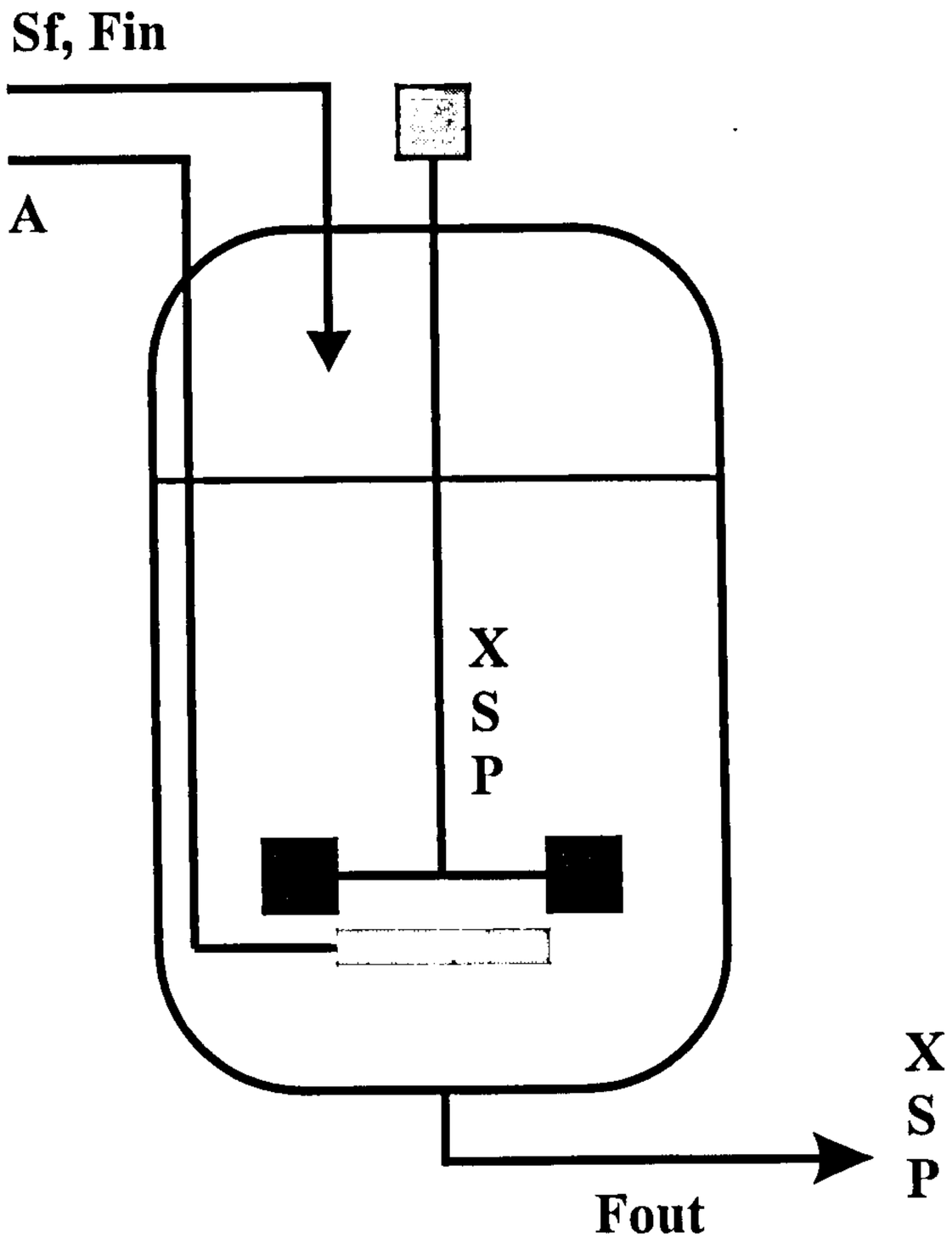


Figure 2-3 Diagram of a fermentation process

Where : X: biomass concentration in the fermenter (g/l)

S: substrate concentration in the fermenter (g/l)

P: product concentration in the fermenter (g/l)

F_{in}: substrate feed rate into the fermenter (l/hr)

F_{out}: culture removal rate (l/hr)

A: air flow rate into the fermenter (l/hr)

S_f: substrate concentration in the feed stream F_{in} (g/l)

Table 2-1 Flow rate for different modes of fermentation.

Mode of Operation	F_{in}	F_{out}
Batch	0	0
Fed-Batch	F_{in}	0
Continuous	$F_{in} = F_{out}$	

In the next section, a brief introduction and review of mathematical modelling for the fermentation processes are presented.

2.3 Modelling of Biotechnological Processes

Modelling procedure is an iterative task as shown in Figure 2-4. It can be separated into two parts as model structure determination and parameter estimation. In the diagram, a priori knowledge of the process can be used to design proper experiments or construct a model structure. Experimental data is then used to estimate parameters of the model. Accuracy of the proposed model is then tested by comparing the model prediction with another set of data. This step is called model validation. If the difference between the model prediction and data is higher than the specified criteria, the proposed model fails and there is a need to go back to modify the structure of the model. The information and knowledge obtained from the previous experiment data, also, leads to a better design of the

next experiments, as well as the better model structure. This iterative task continues until the final model that satisfies the criteria is obtained.

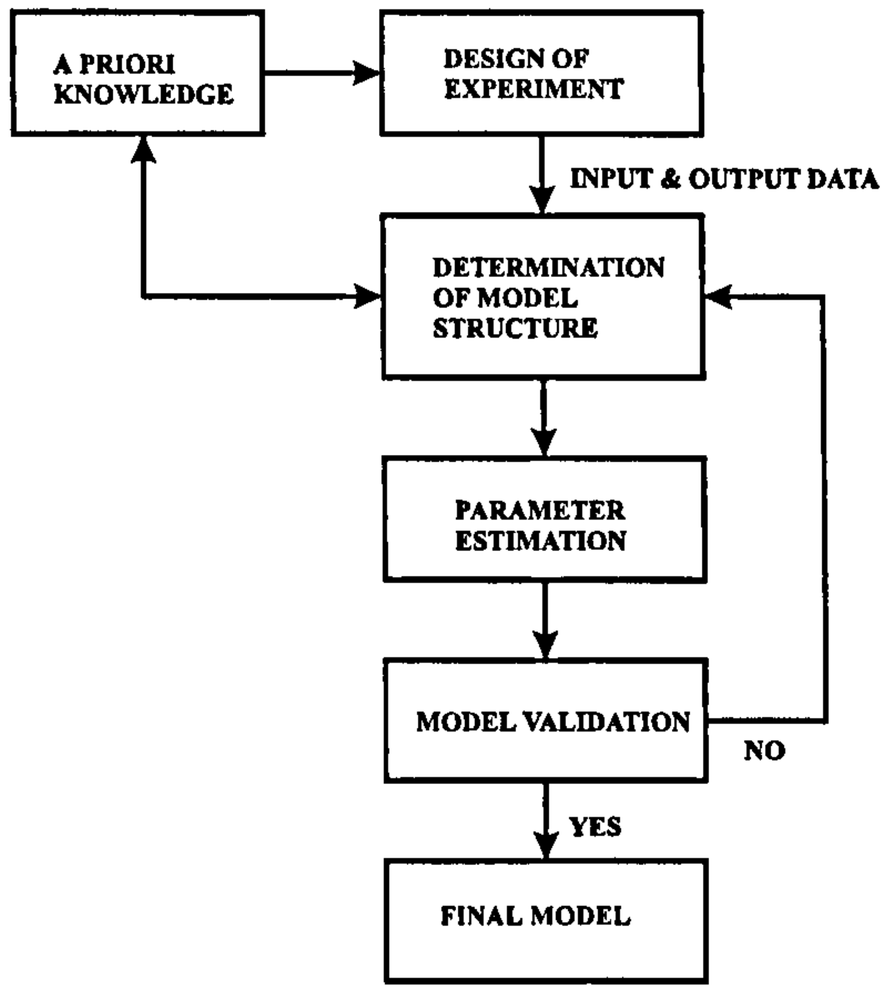


Figure 2-4 Diagram of Modelling Procedure

Bastin *et al.* (1992) divided the problem of model structure determination in fermentation processes into two parts as determination of the number of biological reactions and determination of the kinetic structure of these reaction rates. Model parameters, which are yield coefficients and kinetic parameters, were then identified. As mentioned earlier, a priori knowledge of the process plays an important role in building up the model structure. The different classes of models are served for different purposes. Numerical models, which are used for representing a relationship between input and output, such as Auto Regressive eXogenous (ARX) and Auto Regressive Moving Average eXogenous (ARMAX) are usually sufficient for controlling the processes at pre-specified points. While much more sophisticated ones are needed for process simulation or process design purpose. Since inside microbial mechanisms such as intracellular biochemical pathways in many fermentation processes are not well understood or very complicated, there are many

attempts to use numerical models for representing and controlling fermentation processes (Tang, *et al.*, 1992; Jalel *et al.*, 1992; Mirzai, *et al.*, 1991; Vanichsriratana, *et al.*, 1994). With different degrees of understanding on biological knowledge, two general types of models can be derived.

The so called structured model is used, usually, to study and verify the proposed mechanisms inside the micro-organism, and to perform simulation of the processes. This type of models is developed by using mass balance of intracellular concentrations such as DNA, RNA, enzyme, etc. (Bellgardt, *et al.*, 1989; Palsson and Joshi, 1987). Each cell may also be divided into several compartments (Esener, *et al.*, 1982; Fredrickson, 1976; Harder and Roels, 1982; Roels, 1983), in which reactions and mass transfer of intracellular chemicals are assumed to take place. Structured models need extensive information about internal activity of the cells and is very complicated. It may be for this reason that this type of model has not been used in optimisation of fermentation processes in the literature (Johnson, 1987). There are many articles of structured model available in the literature, for instance, structured model for cell growth and enzyme production by recombinant *Escherichia coli* (Korte, *et al.*, 1991), model of plant cell culture (Bailey and Nicholson, 1989; Gulik, *et al.*, 1993), structured model of *Spirulina platensis* in photobioreactors (Cornet, *et al.*, 1992a; Cornet, *et al.*, 1992b), metabolically structured model of *Thiosphaera pantotropha* (Geraats, *et al.*, 1990), tobacco cell cultures (Hooker and Lee, 1992), lactic acid fermentation (Nielsen, *et al.*, 1991a; Nielsen, *et al.*, 1991b; Nielsen, *et al.*, 1991c), morphologically structured model of filamentous organisms (Nielsen, 1993), penicillin fermentation (Nestaas and Wang, 1983), baker's yeast (Yuan and Bellgardt, 1992), recombinant *Escherichia coli* (Nielsen, *et al.*, 1991d).

The other type of model is called unstructured model. This model assumes that the whole culture is homogeneous and non-segregated. There is also no consideration of the differences between individual cell as far as size, age, chemical composition or morphology are concerned. Therefore the mathematical form of the unstructured model is much simpler than the structured one. Despite its relative simplicity, the unstructured model contains enough information needed for optimisation of the fermentation processes (Cazzador, 1988; Hong, 1986; Lim, *et al.*, 1986; Modak and Lim, 1987; Modak, *et al.*, 1986; Ohno, *et al.*, 1978; Shimizu, *et al.*, 1991; Weigand, *et al.*, 1979). Based on the application potential of the unsturctured model in optimisation of the fermentation processes, the formulation of the unstructured model will be given in the next section and the obtained model will be used for optimisation of the fed-batch fermentation in the following chapters.

2.4 Unstructured Process Modelling for Primary and Secondary Metabolite Production

In this section, the unstructured models for primary and secondary metabolite production are formulated from mass balance equations. Also, the models will be derived for batch, continuous and fed-batch fermentation. However, the emphasis will be given to the fed-batch process since it will be used as the models for process optimisation in this study.

2.4.1 Material balance equations for fermentation processes

The material balance for each component can be written as:

$$\begin{pmatrix}\text{rate of}\\ \text{change}\end{pmatrix} = \begin{pmatrix}\text{rate of}\\ \text{addition}\end{pmatrix} - \begin{pmatrix}\text{rate of}\\ \text{removal}\end{pmatrix} + \begin{pmatrix}\text{rate of}\\ \text{generation}\end{pmatrix} - \begin{pmatrix}\text{rate of}\\ \text{utilisation}\end{pmatrix}$$

Some of the important components in the fermenter are biomass concentration (X), substrate concentration (S), product concentration (P) and culture volume (V). This equation can be applied for all three modes of operation, i.e. batch, fed-batch and continuous. An assumption commonly used is constant culture density. Note also that death rate of the biomass component is assumed to be negligible small and thus omitted from the mass balance equations.

2.4.1.1 Batch fermentation processes

As there is no substrate feeding and culture removal in a batch process, the rate of addition and removal is zero. There is also no change in volume. The cells consume substrate and increase in biomass with the rate of μX. Where μ is the specific growth rate, which is the rate of biomass production per unit biomass and can be written as:

$$\frac{1}{X}\frac{dX}{dt} = \mu$$

Substrate is utilised at the rate of σX. Where σ is the specific substrate usaged rate, which is the rate of substrate utilised or consumed per unit of biomass and can be written as:

$$\frac{1}{X}\frac{dS}{dt} = \sigma$$

Product is produced at the rate of πX. Where π is the specific product formation rate, which is the rate of product formation per unit of biomass and can be written as:

$$\frac{1}{X}\frac{dP}{dt} = \pi$$

The material balance for a batch process is summarised in Table 2-2 and shown in Equation (2-1) to (2-3).

Table 2-2 Summary of material balance in a batch process.

component	rate of change	rate of addition	rate of removal	rate of generation	rate of utilisation
X	dX/dt	0	0	μX	0
S	dS/dt	0	0	0	σX
P	dP/dt	0	0	πX	0
V	0	-	-	-	-

The material balance equations for a batch process are written as:

$$\frac{dX}{dt} = \mu X \qquad (2\text{-}1)$$

$$\frac{dS}{dt} = -\sigma X \qquad (2\text{-}2)$$

$$\frac{dP}{dt} = \pi X \qquad (2\text{-}3)$$

2.4.1.2 Continuous fermentation processes

As mentioned earlier, the log phase can be extended by adding substrate into the fermenter. In continuous fermentation, the culture is taken out at the same rate as substrates are added so there is no change in the culture volume. Biomass is generated at the rate of μX and removed at the rate of DX. Where D is referred to as dilution rate, which is the ratio between the substrate feed rate and the culture volume. Substrate is fed into the system at the rate of DS_f and utilised at the rate of σX. Substrate is also lost by taking out fermentation culture at the rate of DS. Product is formed at the rate of πX and is

taken out at the rate of DP. The material balance for a continuous process is summarised in Table 2-3 and shown in (2-4) to (2-8).

Table 2-3 Summary of material balance in a continuous process.

component	rate of change	rate of addition	rate of removal	rate of generation	rate of utilisation
X	dX/dt	0	DX	μX	0
S	dS/dt	DS_f	DS	0	σX
P	dP/dt	0	DP	πX	0
V	dV/dt	F	F	-	-

The material balance equations are written as:

$$\frac{dX}{dt} = \mu X - DX \qquad (2\text{-}4)$$

$$\frac{dS}{dt} = -\sigma X + D(S_f - S) \qquad (2\text{-}5)$$

$$\frac{dP}{dt} = \pi X - DP \qquad (2\text{-}6)$$

$$\frac{dV}{dt} = 0 \qquad (2\text{-}7)$$

The dilution rate is also defined as:

$$D = \frac{F}{V} \qquad (2\text{-}8)$$

Note also that the continuous fermentation is operated at steady state, in which the following conditions apply:

$$\frac{dX}{dt} = 0$$

$$\frac{dS}{dt} = 0$$

$$\frac{dP}{dt} = 0$$

2.4.1.3 Fed-batch fermentation processes

In a fed-batch fermentation, substrate is fed into the system but no fermentation culture is taken out. Therefore there is no rate of removal in the material balance. However, there is rate of dilution instead. This is due to the amount of substrate fed into the system diluting other components. The material balance for a fed-batch process is summarised in Table 2-4 and shown in Equation (2-9) to (2-13)

Table 2-4 Summary of material balance in a fed-batch process.

component	rate of change	rate of addition	rate of dilution	rate of generation	rate of utilisation
X	dX/dt	0	DX	μX	0
S	dS/dt	DS_f	DS	0	σX
P	dP/dt	0	DP	πX	0
V	dV/dt	F	-	-	-

The material balance equations for a fed-batch process are written as:

$$\frac{dX}{dt} = \mu X - DX \tag{2-9}$$

$$\frac{dS}{dt} = -\sigma X + D(S_f - S) \tag{2-10}$$

$$\frac{dP}{dt} = \pi X - DP \tag{2-11}$$

$$\frac{dV}{dt} = F \tag{2-12}$$

$$D = \frac{F}{V} \tag{2-13}$$

The material balance models for different modes of fermentation have been derived. In the next section, we will consider the equations that are used to describe the specific bioreaction rates (μ, σ and π). These equations usually need biochemical knowledge and process understanding to construct.

2.4.2 Kinetic modelling of primary and secondary metabolite production

As mentioned in the previous sections, the modelling procedures can be generalised into two parts, identification of the model structure and parameter estimation. Model structure can be derived from material and energy balance incorporated with biochemical knowledge which specify the kinetic structure. The model parameters are, then, estimated by choosing the values which correspond to the minimum error between data from the experiment and model prediction. The kinetic parameters may also be found from the published literature. Most parts of fed-batch model structure have been shown above in Equation (2-9) to (2-13). To produce a complete model structure, it is necessary to specify

the kinetic structure of the three main biochemical reactions involved. These reaction rates are specific growth rate (μ), specific substrate consumption rate (σ) and specific product formation rate (π). These reaction rates may be functions of substrate concentration, pH, temperature, etc., however, in this study, it is assumed that these reaction rates are solely functions of the substrate concentration. This does not mean that pH and temperature do not affect the reaction rates but it is assume that they are already regulated at the optimal conditions so that only the effect of substrate concentration on growth and production of micro-organisms in fed-batch fermentation can be examined. There is nevertheless an implicit assumption that there is no time lag of the specific reaction rates from the change of the substrate concentration for these model since the kinetic model or kinetic structure of these specific reaction rates assumes an immediate response to the changing environment. This is due to the fact that the kinetic model is developed from the data at steady state. This immediate response is, commonly, used in chemical reaction kinetics. However, the responding time for the micro-organisms to the change in environment conditions may not be immediately in the real processes. This assumption is worth mentioning here as it is usually failed to be mentioned in the literature. O'Neil and Lyberatos (1990) developed a specific growth rate model, in which time delay was also included.

More than 40 structures for the specific growth rate have been mentioned in the literature (Bastin and Dochain, 1990). The variation of these structures is due to the differences in process characteristics. The most commonly structure used in the literature based on the substrate concentration is the Monod's kinetic (Monod, 1949) and substrate inhibition kinetic. These kinetic equations are:

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{2-14}$$

$$\mu = \frac{\mu_{max} S}{K_s + S + S^2/K_i} \tag{2-15}$$

where μ_{max} is the maximum specific growth rate, K_S and K_i are rate constants. These parameters depend on strain of micro-organisms, type of substrate, etc. The relationship between the specific growth rate and substrate concentration based on the Monod kinetic and substrate inhibition kinetic are shown in Figure 2-5 and Figure 2-6. Note that although both kinetics are often used for representing the relationship between the specific growth rate (μ) and substrate concentration, they can also be used to describe the relationship between other specific rates, such as the specific substrate usage rate (σ) and specific product formation rate (π), and the substrate concentration.

For the Monod type kinetic, the relationship between the specific reaction rate and substrate concentration can be described as a monotonic function. The specific reaction rate increases and finally reaches the maximum specific reaction rate as the substrate concentration increases. The substrate concentration that corresponds to the maximum specific reaction rate must be very high as can be approximated from Equation (2-14). Note that the maximum specific reaction rate is the highest specific reaction rate and can not be increased higher than this value. (The maximum specific reaction rate is specific to particular micro-organisms strain, substrate, etc.) This maximum specific reaction rate can therefore be seen as a constraint imposed on the reaction rate. The physical meaning of K_S can also be obtained from Equation (2-14) as the substrate concentration for which the specific reaction rate equals half of the maximum specific reaction rate. The substrate

considered in this case is the limiting substrate because the reaction rates depend on the amount of substrate concentration.

The relationship between the specific reaction rate and substrate concentration for the substrate inhibition type kinetic can be described as a nonmonotonic function. It is shown in Figure 2-6 that the specific reaction rate increases as the substrate concentration increases until substrate concentration reaches a certain level after which the specific reaction rate starts decreasing. This is because too much substrate concentration inhibits the specific reaction rate. The substrate considered in this case is, therefore, the limiting substrate, which also has the inhibition property. This type of kinetic is more realistic than the Monod type because it can represents the catabolite repression or glucose effect in the fermentation processes.

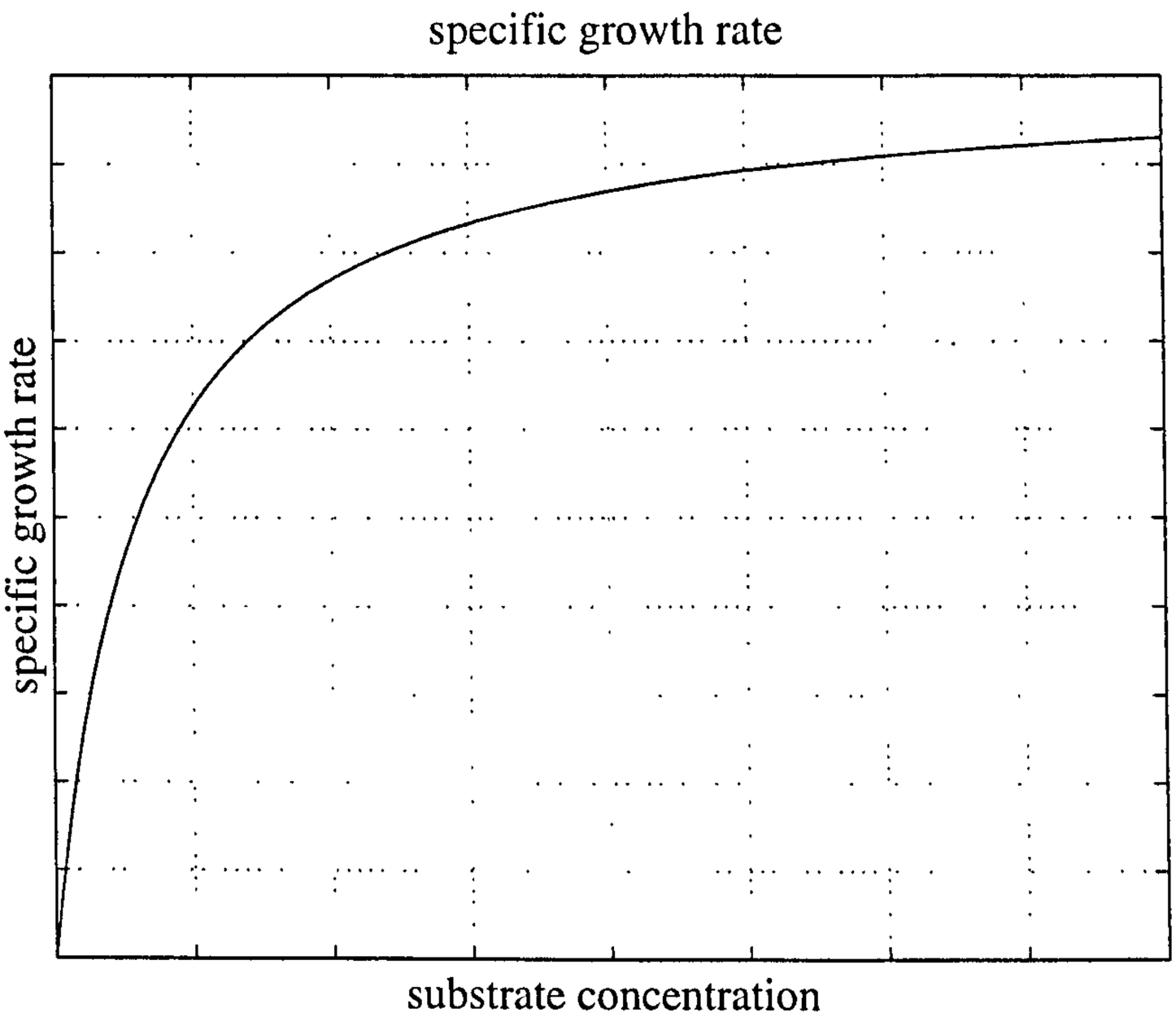


Figure 2-5 Relation between specific growth rate (μ) and substrate concentration (S) in Monod kinetic

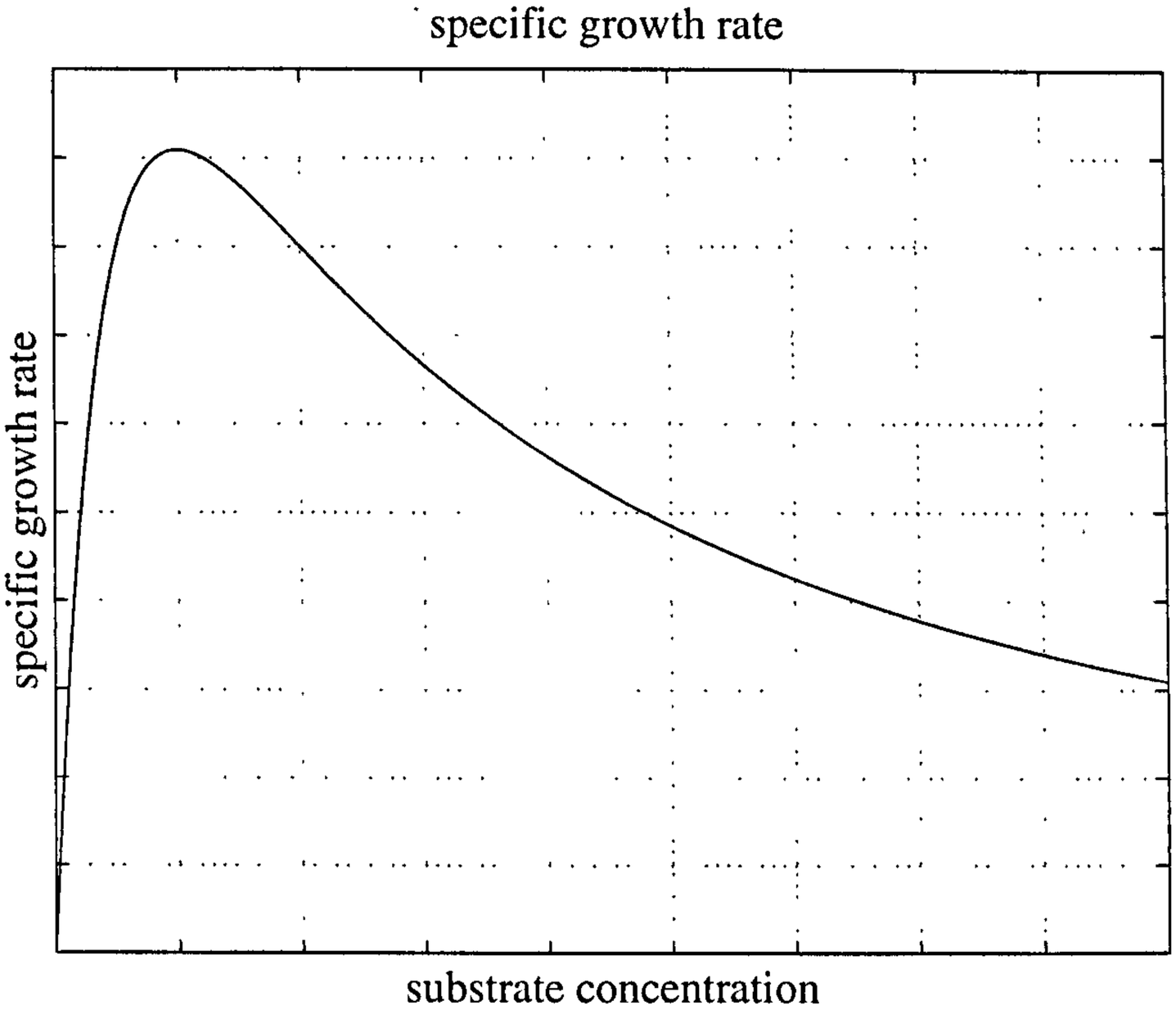


Figure 2-6 Relation between specific growth rate (μ) and substrate concentration (S) in substrate inhibition kinetic

There are, also, many expressions for the specific substrate consumption rate. The most commonly used is in the form of Eqaution (2-16), in which Y_{xs} is the yield of biomass from substrate. It is, actually, the conversion factor to show how many units of biomass can be generated per unit of substrate. The specific substrate consumption rate (σ) is therefore related to the biomass production and the specific growth rate (μ), and can be written as:

$$\sigma = -\frac{1}{Y_{xs}}\mu \qquad (2\text{-}16)$$

For the specific product formation rate, a well known 'Luedeking - Piret' equation (Luedeking and Piret, 1959) as in Equation (2-17) is often used. The model assumes two stages of product formation, one during growth phase and the other during stationary phase. The values of coefficients, α and β, therefore, depend on the type of the desired

product. To produce a primary metabolite produced during the growth phase, the coefficient β will be zero. While for a secondary metabolite, produced in the stationary phase, the coefficient α will be zero.

$$\pi = \alpha\mu + \beta \qquad (2\text{-}17)$$

As stated earlier, these structures of the models for the specific reaction rate (μ, σ, π) are based on the biochemical knowledge of the processes. The material balance models in the previous section incorporated with the kinetic structures of the bioreaction rates become the complete models, in which the unknown coefficients (such as Y_{xs}, K_s, K_i, α, β, etc.) are left to be estimated from the experimental data.

In general, a model would be limited only by the knowledge and imagination and therefore many different model structures can be obtained. Bosnjak *et al.* (1978) used Luedeking-Piret equation for erythromycin biosynthesis from *Streptomyces erythreus*. They also used three different models as shown in Equation (2-18) to (2-20) to describe the microbial growth phase in pellet forms, stationary phase and decline phase respectively (Bosnjak, *et al.*, 1979; Bosnjak, *et al.*, 1981; Bosnjak, *et al.*, 1985). Where k, k_1, k_2, k_3 are constant coefficients.

$$X^{1/3} = k\,t + X_0^{1/3} \qquad (2\text{-}18)$$

$$\frac{dX}{dt} = k_1 X^{2/3} - k_2 X \qquad (2\text{-}19)$$

$$\frac{dX}{dt} = k_1 X^{2/3} - k_2 X - k_3 X t \qquad (2\text{-}20)$$

For penicillin fermentation, Bajpai and Reuβ (1980) used Contois kinetic as shown in Equation (2-21) to describe the specific growth rate. The specific production rate was

modelled by a substrate inhibition kinetic as shown in Equation (2-22). The substrate utilisation model was, then, obtained by assuming constant yield and maintenance coefficient to the growth and production as shown in Equation (2-23).

$$\mu = \frac{\mu_{max} S}{K_x X + S} \tag{2-21}$$

$$\pi = \frac{\mu_p S}{K_p + S(1 + S/K_i)} \tag{2-22}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{xs}}\frac{dX}{dt} - \frac{1}{Y_{ps}}\frac{dP}{dt} - m_s X \tag{2-23}$$

where

μ_{max} : maximum specific growth rate (hr^{-1})

μ_p : maximum specific production rate (hr^{-1})

K_x : biomass coefficient constant (-)

K_p : product coefficient constants (g/l)

K_i : substrate inhibition constant (g/l)

Y_{xs} : yield of biomass from substrate (g biomass/g substrate)

Y_{ps} : yield of product from substrate (g product/g substrate)

2.5 Model Representation of Primary and Secondary Metabolite Production in Fed-Batch Mode

In this section, the models for primary and secondary metabolite production in fed-batch mode are presented. These models are based on material balance of fed-batch fermentation

shown in Equations (2-9) to (2-13) incorporated with kinetic structures presented in the previous section.

2.5.1 Primary metabolite production model

It was mentioned in Section 2.1 that biomass production itself is also a primary product. From material balance equation of biomass, model for change in biomass concentration can be obtained in Equation (2-24) and model for change of substrate concentration can be obtained in Equation (2-25) in which Equation (2-16) is used for the specific substrate consumption rate.

$$\frac{dX}{dt} = \mu X - DX \tag{2-24}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{xs}}\mu X + D(S_f - S) \tag{2-25}$$

$$\frac{dV}{dt} = F \tag{2-26}$$

$$D = \frac{F}{V} \tag{2-27}$$

2.5.2 Secondary metabolite production model

As in the primary metabolite production in the previous section, models for change in biomass and substrate concentration are shown in Equation (2-28) and (2-29). For change in product formation, the model in Equation (2-30) is used for the secondary metabolite production.

$$\frac{dX}{dt} = \mu X - DX \tag{2-28}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{xs}}\mu X + D(S_f - S) \tag{2-29}$$

$$\frac{dP}{dt} = \pi X - DP \tag{2-30}$$

$$\frac{dV}{dt} = F \tag{2-31}$$

$$D = \frac{F}{V} \tag{2-32}$$

2.5.3 Kinetic structure of metabolite production

The kinetic structures for the specific growth rate (μ) and specific product formation rate (π) will be represented by the Monod and substrate inhibition type kinetics because of their common and widely used in fermentation kinetic modelling. However, it will be shown in Chapter 3 that for the Monod type kinetic, the fed-batch fermentation should operate in batch mode, hence the substrate inhibition kinetic will be mostly used for the kinetic structures in this thesis and also for the comparison simulation in Chapter 5.

The 'Luedeking - Piret' kinetic in Equation (2-17) will not be used as the kinetic structure for the specific product formation rate in this study for the following reasons.

1. There is a linear relationship between the specific growth rate and the specific product formation rate. If both α and β are not zero, the biomass and product would be produced at the same time and have the same production pattern.

2. If α becomes zero as in the secondary metabolite production case, the specific product formation rate would be constant (β) for the whole batch. This can not really represent the secondary metabolite production as the product formation does not begin from the start of the fermentation.

It has also been shown by Quinlan (1986) that, the specific production rate (π) for the secondary metabolite ($\beta \neq 0$) in this model does not depend on the substrate concentration. Therefore the product would be produced by this model even after the substrate has been depleted from the fermenter, which is not realistic.

2.6 Modelling Analysis

The following constraints are imposed (implicitly or explicitly) on the mathematical model of the fed-batch fermentation processes.

- Growth rate: The growth rate of micro-organisms is limited by the maximum specific growth rate.
- Product formation rate: The product formation rate is limited by the maximum specific product formation rate.
- Substrate concentration. The substrate concentration in the fermenter can not exceed the concentration of substrate in the feed stream (S_f).
- Feed rate. The substrate feed rate is constrained by maximum and minimum feed rates, which are the physical constraints of the process.

In the next chapter, an optimisation of fed-batch fermentation using substrate feed rate is introduced. As an objective function and optimisation procedure are established, the fermentation model presented in this chapter will be used as equality constraints for this optimisation problem.

Chapter 3. Open Loop Optimal Control of Fermentation Processes

3.0 Introduction

Calculus of variations or Lagrange optimisation provides a natural approach for process optimisation. It has been used to optimise many fermentation processes in the literature (Cazzador, 1988; Hong, 1986; Lim, *et al.*, 1986; Modak and Lim, 1987; Ohno, *et al.*, 1978; Shimizu, *et al.*, 1991; Weigand, *et al.*, 1979). The method results in a two-point boundary-value problem in which, due to the nonlinear nature of the process, an iterative search is needed to solve the optimisation problem and thus results in an open loop control algorithm. In this chapter, the calculus of variations for optimising the fermentation processes used in the literature is formulated and then applied to primary and secondary metabolite production processes.

The resulting optimal solution consists of time sequence of maximum, minimum and singular feed rates. The physical meaning of these feed rates sequence can be interpreted by using a knowledge gained from analysis of the condition where the singular period occurs.

3.1 Calculus of Variations and Open Loop Optimal Feed Rate Control

The general fed-batch process model (Equation (2-9) to (2-13)) from Chapter 2 is written here for convenience as Equation (3-1) to (3-5):

$$\frac{dX}{dt} = \mu X - DX \tag{3-1}$$

$$\frac{dS}{dt} = -\sigma \mu X + D(S_f - S) \tag{3-2}$$

$$\frac{dP}{dt} = \pi X - DP \tag{3-3}$$

$$\frac{dV}{dt} = F \tag{3-4}$$

$$D = F/V \tag{3-5}$$

One of the main objectives of the fermentation process is to produce as much desired product as possible under production-time constraints. Process optimisation using the calculus of variations (Bryson and Ho, 1975; Kirk, 1970; Noton, 1972; Ramirez, 1994) is therefore used here to achieve this purpose. We, firstly, define an objective function, which is a function of biomass and/or product at the final operating time:

$$J(F) = f\left(X(t_f), P(t_f)\right) \tag{3-6}$$

Feed rate (F) is a control input determined to maximise this objective function. With the objective function in (3-6) and process model in Equation (3-1) to (3-4), the corresponding Hamiltonian equation (refer to (Bryson and Ho, 1975; Kirk, 1970; Noton, 1972; Ramirez, 1994) for details) can then be written as:

$$H = \lambda_X \frac{dX}{dt} + \lambda_S \frac{dS}{dt} + \lambda_P \frac{dP}{dt} + \lambda_V \frac{dV}{dt}$$

or

$$H = \lambda_X (\mu X - DX) + \lambda_S \left(-\sigma \mu X + D(S_f - S)\right) + \lambda_P (\pi X - DP) + \lambda_V F \tag{3-7}$$

and the costate equations:

$$\dot{\lambda}_X = -\frac{\partial H}{\partial X} = -\lambda_X(\mu - D) + \sigma\lambda_S\mu - \lambda_P\pi \tag{3-8}$$

$$\dot{\lambda}_S = -\frac{\partial H}{\partial S} = -\lambda_X X\mu' + \sigma\lambda_S X\mu' + D\lambda_S - \lambda_P X\pi' \tag{3-9}$$

$$\dot{\lambda}_P = -\frac{\partial H}{\partial P} = -\lambda_P D \tag{3-10}$$

$$\dot{\lambda}_V = -\frac{\partial H}{\partial V} = -\frac{F\lambda_X X}{V^2} + \frac{F\lambda_S}{V^2}(S_f - S) - \frac{F\lambda_P P}{V^2} \tag{3-11}$$

Where ′ indicates the first derivative with respect to substrate concentration and λ_X, λ_S, λ_P, λ_V are the costates for biomass concentration (X), substrate concentration (S), product concentration (P) and culture volume (V) respectively.

The transversality or final conditions can also be written as:

$$\lambda_X(t_f) = \frac{\partial J}{\partial X_{tf}}$$

$$\lambda_P(t_f) = \frac{\partial J}{\partial P_{tf}}$$

Where X_{tf} and P_{tf} are the biomass and product concentration at the final operating time.

$$X_{tf} = X(t_f)$$

$$P_{tf} = P(t_f)$$

Additionally, the substrate concentration at the final time is not fixed, therefore,

$$\lambda_S(t_f) = 0$$

Once these conditions have been established, the process optimisation using the substrate feed rate (F) can then be determined in the next section.

3.1.1 Optimal feed rate profile

The optimal feed rate profile that would optimise the fermentation process is determined in this subsection. Since there are constraints on feed rate, which is the control variable, the Pontryagin's Maximum principle is therefore applied in this case. The Hamiltonian Equation (3-7) is rearranged as:

$$H = (\lambda_X \mu - \lambda_S \sigma \mu + \lambda_P \pi) X + \Psi F$$

where

$$\frac{\partial H}{\partial F} = -\frac{\lambda_X X}{V} + \lambda_V + \frac{\lambda_S (S_f - S)}{V} - \frac{\lambda_P P}{V} = \Psi \qquad (3\text{-}12)$$

It is stated by the Maximum principle that the Hamiltonian must be maximised at all times over all possible feed rate (F). Therefore the optimal feed rate is determined by the sign of Ψ as followed:

if $\Psi < 0$ then $F = 0$

if $\Psi > 0$ then $F = F_{max}$

However, it is possible that Ψ can become zero at some period of time during the process operation and the Maximum principle will, thus, fail to provide an appropriate feed rate in this case. This situation usually happens when the process model is linear in control variables and so is the Hamiltonian. This characteristic is well known in the literature as a 'singular problem'. It has also been shown in (Ramirez, 1994) that, for a time optimal

control of a linear system, the singular control would occur only to a system that is uncontrollable.

The corresponding feed rate for this singular period is referred to as a singular feed rate (F_{sing}) and since Ψ is zero over the singular period, all of its time derivatives are also zero over this period; i.e.

$$\frac{d^k}{dt^k}\Psi = 0 \; ; \; k = 1,2,3,\ldots \tag{3-13}$$

With this condition applied, the singular feed rate can be determined by repeatedly differentiating Ψ until feed rate (F) reappears in the time derivative equation of Ψ. As the patterns of feed rate are known to consist of maximum, minimum and singular feed rates, the optimisation problem can therefore be reduced to determine the optimal sequence of these feed rates and the corresponding switching times. The computational algorithms for this purpose can be found in (Van Impe, *et al.*, 1992; Lim, *et al.*, 1986).

3.2 Optimal Control of Primary and Secondary Metabolite production

The optimisation scheme obtained from the previous section, which produces an optimal feed rate profile will be applied to specific cases of primary and secondary metabolite production processes in this section.

3.2.1 Primary metabolite production

It is known from Chapter 2 that primary metabolites are produced during the microbial growth and the amount of biomass can be used as an indication of the primary metabolite production. The biomass production is, therefore, used in this study as an example of the

primary metabolite production process. The process model is that given in Equation (2-24) to (2-27):

$$\frac{dX}{dt} = \mu X - DX \tag{3-14}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{xs}}\mu X + D(S_f - S) \tag{3-15}$$

$$\frac{dV}{dt} = F \tag{3-16}$$

$$D = F/V \tag{3-17}$$

The aim for this primary metabolite (biomass) production is to maximise the biomass concentration (X) at the final operating time using the substrate feed rate (F). This aim can be transformed into an objective function as:

$$J(F) = X(t_f) - \varepsilon \int_{t_0}^{t_f} dt \tag{3-18}$$

Where ε is the cost factor per unit of operating time. The Hamiltonian equation for this process can then be written as:

$$H = -\varepsilon + \lambda_X(\mu X - DX) + \lambda_S\left(-\frac{1}{Y_{xs}}\mu X + D(S_f - S)\right) + \lambda_V F \tag{3-19}$$

and the costate equations:

$$\dot{\lambda}_x = -\frac{\partial H}{\partial X} = -\lambda_X(\mu - D) + \frac{1}{Y_{xs}}\lambda_S \mu \tag{3-20}$$

$$\dot{\lambda}_s = -\frac{\partial H}{\partial S} = -\lambda_X X\mu' + \frac{1}{Y_{xs}}\lambda_S X\mu' + D\lambda_S \tag{3-21}$$

$$\dot{\lambda}_V = -\frac{\partial H}{\partial V} = -\frac{F\lambda_X X}{V^2} + \frac{F\lambda_S}{V^2}(S_f - S) \tag{3-22}$$

The transversality or final conditions can also be written as:

$$\lambda_x(t_f) = \frac{\partial J}{\partial X_{tf}} = 1$$

and

$$\lambda_s(t_f) = 0$$

The optimal control sequence is then calculated from Equation (3-23) in which the sign of Ψ is used to indicate the period of maximum, minimum or singular feed rate.

$$\frac{\partial H}{\partial F} = -\frac{\lambda_X X}{V} + \lambda_V + \frac{\lambda_S(S_f - S)}{V} = \Psi \tag{3-23}$$

if $\Psi < 0$ then $F = 0$

if $\Psi > 0$ then $F = F_{max}$

if $\Psi = 0$ then $F = F_{sing}$

The singular feed rate can be determined by differentiating Equation (3-23) until feed rate (F) reappears in the equation. The first derivative of (3-23) is shown as:

$$\frac{d\Psi}{dt} = 0 = \frac{\lambda_S \mu' X(S_f - S)}{V Y_{xs}} - \frac{\lambda_X \mu' X(S_f - S)}{V} \tag{3-24}$$

which implies that

$$\mu' = \frac{\partial \mu}{\partial S} = 0 \tag{3-25}$$

or

$$\frac{\lambda_S}{Y_{xs}} - \lambda_X = 0 \qquad (3\text{-}26)$$

It can be proved by contradiction that Equation (3-26) is not satisfied during the singular period. To illustrate this, it is assumed that Equation (3-26) is satisfied during the singular period. The Hamiltonian equation (3-19) during the singular period is:

$$H = -\varepsilon + \lambda_X \mu X - \lambda_S \frac{1}{Y_{xs}} \mu X \qquad (3\text{-}27)$$

Since the final operating time for this process is not fixed (free final time problem), the Hamiltonian is constant and equals to zero. This condition is not valid if Equation (3-26) is satisfied. Therefore Equation (3-25) is the only necessary condition for the singular period to happen in this process.

To determine the singular feed rate, Equation (3-23) is differentiated again. The second derivative of Ψ is:

$$\frac{d^2\Psi}{dt^2} = 0 \qquad (3\text{-}28)$$

By using Equation (3-14) to (3-16), (3-20), (3-21) and (3-25), the singular feed rate can be derived from Equation (3-28) as:

$$F_{\text{sing}} = \frac{\mu X V}{Y_{xs}\left(S_f - S\right)} \qquad (3\text{-}29)$$

The substrate concentration (S) during the singular period will be called "the singular substrate concentration (S_{sing})" and can be obtained by solving Equation (3-25). Equation (3-29) is then written as:

$$F_{\text{sing}} = \frac{\mu X V}{Y_{xs}\left(S_f - S_{\text{sing}}\right)} \qquad (3\text{-}30)$$

The singular feed rate can be interpreted as a regulator control law which maintains the substrate concentration constant at value S_{sing}. In the above equation, ($\mu XV/Y_{xs}$) is the amount of substrate that is needed to produce biomass, and (S_f-S_{sing}) is the amount of substrate that is provided to produce biomass after keeping the substrate concentration constant at S_{sing}. The ratio of these two values results in a desired feed rate that will control the substrate concentration at this singular level (S_{sing}).

It can also be compared with the material balance of substrate concentration in Equation (3-15), in which Equation (3-30) can be obtained under a condition that the substrate concentration is to be kept constant.

There are many structures for the specific growth rate (μ) as mentioned in Chapter 2. However, only the Monod and substrate inhibition kinetic are considered here because of their most common and wide use for representing the specific growth rate (μ).

3.2.1.1 Monod kinetic

The Monod type kinetic for the specific growth rate (μ) is in the following structure:

$$\mu = \frac{\mu_{max} S}{K_s + S}$$

The graphical representation of the relationship between the specific growth rate (μ) and substrate concentration (S) is shown in Figure 2-5 in Chapter 2. In this case, there is no singular period as the substrate concentration (S) that will satisfy Equation (3-25) is not finite (The substrate concentration would be at infinity). The substrate concentration will therefore be kept as high as possible in order to increase the biomass growth rate (see the relationship between the specific growth rate (μ) and the substrate concentration (S) in Figure 2-5). The optimal feed rate sequence in this case consists of only the maximum and

minimum feed rates. The maximum feed rate starts first from the beginning of the batch until the reactor is full. It is then followed by the minimum feed rate (no substrate is fed into the fermenter) until the process is finished (the final conditions or requirements are satisfied). The maximum and minimum feed rates are also decided by the sign of Ψ in Equation (3-23).

With this kinetic structure and the corresponding optimal feed rate sequence, the process operation is, in fact, equivalent to a batch fermentation, as the fermenter is filled up from the beginning of the batch and the process then continues without any substrate feeding until the end of the batch.

3.2.1.2 Substrate inhibition kinetic

The widely use of the substrate inhibition kinetic is due to its ability to represent the catabolite repression or glucose effect behaviour in the fermentation process. The relationship between the specific growth rate (μ) and substrate concentration (S) is in the following structure:

$$\mu = \frac{\mu_{max} S}{K_s + S + S^2/K_i}$$

The graphical representation of the specific growth rate (μ) and substrate concentration (S) is shown in Figure 2-6 in Chapter 2. In this case, the singular substrate concentration (S_{sing}) can be obtained by solving Equation (3-25) which results in,

$$S_{sing} = \sqrt{K_s K_i} \qquad (3\text{-}31)$$

Therefore, the substrate concentration (S) in the reactor is maintained at this constant value (S_{sing}) during the singular control period. It is also worth mentioning that the singular

substrate concentration (S_{sing}) is in fact the concentration that maximises the specific growth rate ($\mu'=0$). The singular feed rate from (3-29) then becomes:

$$F_{sing} = \frac{\mu X V}{Y_{xs}\left(S_f - \sqrt{K_s K_i}\right)} \tag{3-32}$$

In the next section, we will consider the feed rate optimisation of the secondary metabolite production process.

3.2.2 Secondary metabolite production

For a secondary metabolite production process, the process model from Chapter 2 (Equation (2-28) to (2-32)) are written here as:

$$\frac{dX}{dt} = \mu X - D X \tag{3-33}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{xs}}\mu X + D\left(S_f - S\right) \tag{3-34}$$

$$\frac{dP}{dt} = \pi X - D P \tag{3-35}$$

$$\frac{dV}{dt} = F \tag{3-36}$$

$$D = F/V \tag{3-37}$$

The objective function here is to maximise the secondary metabolite product (P) at the final time (t_f) and can be stated as:

$$J(F) = P\left(t_f\right) - \varepsilon\int_{t_0}^{t_f} dt \tag{3-38}$$

The Hamiltonian equation for this process can then be written as:

$$H = -\varepsilon + \lambda_X(\mu X - DX) + \lambda_S\left(-\frac{1}{Y_{xs}}\mu X + D(S_f - S)\right) + \lambda_P(\pi X - DP) + \lambda_V F \tag{3-39}$$

or

$$H = -\varepsilon + \left(\lambda_X \mu - \frac{\lambda_S}{Y_{xs}}\mu + \lambda_P \pi\right)X + \left(-\frac{\lambda_X X}{V} + \lambda_V + \frac{\lambda_S(S_f - S)}{V} - \frac{\lambda_P P}{V}\right)F \tag{3-40}$$

and the costate equations:

$$\dot{\lambda}_X = -\frac{\partial H}{\partial X} = -\lambda_X(\mu - D) + \frac{1}{Y_{xs}}\lambda_S \mu - \lambda_P \pi \tag{3-41}$$

$$\dot{\lambda}_S = -\frac{\partial H}{\partial S} = -\lambda_X X\mu' + \frac{1}{Y_{xs}}\lambda_S X\mu' + D\lambda_S - \lambda_P X\pi' \tag{3-42}$$

$$\dot{\lambda}_P = -\frac{\partial H}{\partial P} = \lambda_P D \tag{3-43}$$

$$\dot{\lambda}_V = -\frac{\partial H}{\partial V} = -\frac{F\lambda_X X}{V^2} + \frac{F\lambda_S}{V^2}(S_f - S) - \frac{F\lambda_P P}{V^2} \tag{3-44}$$

The transversality or final conditions can be written as:

$$\lambda_X(t_f) = 0 \tag{3-45}$$

$$\lambda_S(t_f) = 0 \tag{3-46}$$

$$\lambda_P(t_f) = \frac{\partial J}{\partial P_{tf}} = 1 \tag{3-47}$$

The optimal control sequence is then calculated from Equation (3-48) in which the sign of Ψ is used to indicate the period of maximum, minimum or singular feed rate.

$$\frac{\partial H}{\partial F} = -\frac{\lambda_X X}{V} + \lambda_v + \frac{\lambda_S (S_f - S)}{V} - \frac{\lambda_P P}{V} = \Psi \qquad (3\text{-}48)$$

if $\Psi < 0$ then $F = 0$

if $\Psi > 0$ then $F = F_{max}$

if $\Psi = 0$ then $F = F_{sing}$

During the singular period ($\Psi = 0$), the singular feed rate is determined by differentiating of Equation (3-48) until feed rate (F) reappears in the equation. The first derivative of Ψ is:

$$\frac{d\Psi}{dt} = 0 = \frac{\lambda_S \mu' X (S_f - S)}{VY} - \frac{\lambda_X \mu' X (S_f - S)}{V} - \frac{\lambda_P \pi' X (S_f - S)}{V} \qquad (3\text{-}49)$$

which implies that,

$$\frac{\lambda_S \mu'}{Y} - \lambda_X \mu' - \lambda_P \pi' = 0 \qquad (3\text{-}50)$$

And the second derivative of Ψ is,

$$\frac{d^2\Psi}{dt^2} = 0 \qquad (3\text{-}51)$$

The singular feed rate can then be obtained from Equation (3-51) using Equations (3-33) to (3-36), (3-41) to (3-44) and (3-50) as:

$$F_{sing} = \frac{V}{(S_f - S)} \left[\frac{\mu X}{Y_{xs}} + \frac{\mu' \left(\frac{\lambda_S}{Y_{xs}} \mu - \lambda_X \mu - \lambda_P \pi \right)}{\left(\frac{\lambda_S}{Y_{xs}} \mu'' - \lambda_X \mu'' - \lambda_P \pi'' \right)} \right] \qquad (3\text{-}52)$$

Where $'$ and $''$ are the first and second derivatives with respect to the substrate concentration. Equation (3-52) is in fact a general form for the singular feed rate both in the primary and secondary metabolite production. In the singular period of the primary

metabolite process, the conditions in (3-25) and (3-31) when applied to (3-52) result in the singular feed rate in (3-32). Comparing Equation (3-52) to the mass balance equation of substrate concentration in Equation (3-34), the following equation is obtained:

$$\frac{dS_{\text{sing}}}{dt} = \frac{\mu'\left(\frac{\lambda_S}{Y_{xs}}\mu - \lambda_X\mu - \lambda_P\pi\right)}{\left(\frac{\lambda_S}{Y_{xs}}\mu'' - \lambda_X\mu'' - \lambda_P\pi''\right)} \tag{3-53}$$

Equation (3-53) is the singular substrate concentration trajectory during the singular period. To understand the meaning of the singular feed rate, the analysis from the primary metabolite case in the previous section can also be used here by separating the singular feed rate into two parts - the substrate consumption part and substrate providing part as shown in the following:

$$\text{substrate consumed} = V\left[\frac{\mu X}{Y_{xs}} + \frac{\mu'\left(\frac{\lambda_S}{Y_{xs}}\mu - \lambda_X\mu - \lambda_P\pi\right)}{\left(\frac{\lambda_S}{Y_{xs}}\mu'' - \lambda_X\mu'' - \lambda_P\pi''\right)}\right]$$

$$\text{substrate provided} = \left(S_f - S_{\text{sing}}\right)$$

The singular feed rate for the secondary metabolite process can therefore be seen as feed rate that maintains substrate concentration following the trajectory in Equation (3-53).

During the singular period, Equation (3-48) equals zero:

$$-\frac{\lambda_X X}{V} + \lambda_V + \frac{\lambda_S\left(S_f - S\right)}{V} - \frac{\lambda_P P}{V} = \Psi = 0 \tag{3-54}$$

Since this is a free time problem (the final operating time is not fixed), the Hamiltonian (H) becomes zero and Equation (3-40) during the singular period (Equation (3-54)) then becomes;

$$H = -\varepsilon + \left(\lambda_X \mu - \frac{\lambda_S}{Y_{xs}}\mu + \lambda_P \pi\right) X = 0 \tag{3-55}$$

or

$$X = -\frac{\varepsilon}{\left(\frac{\lambda_S}{Y_{xs}}\mu - \lambda_X \mu - \lambda_P \pi\right)} \tag{3-56}$$

Substituting Equation (3-50), which is the condition for the singular period into Equation (3-56) results in;

$$X = -\frac{\mu'\varepsilon}{(\pi'\mu - \pi\mu')} \tag{3-57}$$

Equation (3-57) shows the relationship between the substrate and biomass concentration at the different operating cost factor (ε) during the singular period. The profile of substrate concentration (Equation (3-53)) during the singular period, and the singular feed rate (Equation (3-52)) in this case become:

$$\frac{dS_{\text{sing}}}{dt} = \frac{\mu'(\pi'\mu - \pi\mu')}{(\pi'\mu'' - \pi''\mu')} \tag{3-58}$$

$$F_{\text{sing}} = \frac{V}{(S_f - S_{\text{sing}})}\left(\frac{\mu X}{Y_{xs}} + \frac{\mu'(\pi'\mu - \pi\mu')}{(\pi'\mu'' - \pi''\mu')}\right) \tag{3-59}$$

The singular substrate concentration is not constant as in the primary metabolite process, but follows the profile in Equation (3-58). This circumstance is, however, different in another condition where the given objective function does not take the operating cost into account (ε = 0). The objective function then becomes:

$$J(F) = P(t_f) \tag{3-60}$$

Following the same procedure, the Hamiltonian equation in (3-40) during the singular period, given the objective function (3-60), becomes:

$$H = \left(\lambda_X \mu - \frac{\lambda_S}{Y_{xs}}\mu + \lambda_P \pi\right)X = 0$$

or

$$\frac{\lambda_S \mu}{Y_{xs}} - \lambda_X \mu - \lambda_P \pi = 0 \qquad (3\text{-}61)$$

And the singular feed rate in this case (from Equation (3-52)) becomes:

$$F_{\text{sing}} = \frac{V}{\left(S_f - S_{\text{sing}}\right)}\frac{\mu X}{Y_{xs}} \qquad (3\text{-}62)$$

Equation (3-62) shows that the substrate concentration is kept constant at S_{sing} during the singular period. The singular substrate concentration (S_{sing}) can be obtained from combining Equation (3-50) and (3-61) which results in:

$$\lambda_P(\mu\pi' - \pi\mu') = 0$$

Since λ_P is not zero, this implies,

$$(\mu\pi' - \pi\mu') = 0$$

or

$$\frac{d(\pi/\mu)}{dS} = 0 \qquad (3\text{-}63)$$

The substrate concentration is therefore kept constant at the level which maximises the ratio of the specific product formation rate over the specific growth rate. Note that this level of substrate concentration is not necessarily the level that maximises the product formation rate (i.e. $\pi' = 0$). With the ratio of high production rate over low growth rate, the

process will take long operating time due to the slow growth rate and result in high product concentration at the end of the batch. This condition is obtained from the fact that we have considered only maximising product concentration and did not put the cost of operating time into account. This can be considered as an ideal condition for maximising secondary metabolite production. However, most of the process would operate under some production-time constraint considered earlier and too long operating time might not be applicable in industry.

In the next section, The optimal feed rate profiles for both primary and secondary metabolite production processes are analysed for process understanding. The relationship between the feed rate sequence is also explained.

3.3 Interpretation of Optimal Feed Rate Control in Fermentation Processes

Optimal feed rate profiles have been used extensively for optimising fermentation processes in the literature. (Cazzador, 1988; Hong, 1986; Lim, *et al.*, 1986; Modak and Lim, 1987; Modak, *et al.*, 1986; Ohno, *et al.*, 1978; Park and Ramirez, 1988; Shimizu, *et al.*, 1991; Weigand, *et al.*, 1979). The optimal feed rate profile has been shown in the previous section to consist of combinations of minimum, maximum and singular feed rates.

As the method usually results in the pre-determined feed rate profile based on the sign of Ψ (refer to Equation (3-12)), the physical meaning of these feed rate sequences on the fermentation processes can not be seen clearly from the process operational point of view because operators would be given only the pre-specified sequence of feed rate to operate on the process without any knowledge of what is happening inside.

The analysis is therefore carried out to understand the effect of the optimal feed rate profile on the process. The meaning of singular period in the fermentation processes will be established first since the optimal path in this period is not affected by the feed rate constraints. It will be extended later to cover the meaning and effect of maximum and minimum feed rate on the fermentation processes.

During the singular period, the Hamiltonian is maintained at the maximum by keeping substrate concentration at the singular level (or profile). Note that the singular feed rate (F_{sing}) is used to keep the substrate concentration at this trajectory and therefore not necessarily constant.

As the singular period is an interval that optimises the process by controlling the substrate concentration at the optimal level, the meaning of the maximum and minimum feed rate outside the singular period become clear. These feed rates are used to shift the substrate concentration from the initial condition that is not optimal to the optimal one and then start the singular period. Therefore, if the initial substrate concentration in the reactor starts at the optimal level, the process would start with the singular feed rate and continue until it reaches the constraints. This can be seen when the maximum feed rate can not provide adequate substrate to maintain the optimal substrate concentration (maximum feed rate constraint) or the culture volume reaches the maximum (maximum volume constraint), which also results in the minimum feed rate (F = 0). The optimality of feed rate at the maximum and minimum is due to these constraints and follows the Maximum principle.

3.4 Discussion

There are two problems arising from applying the open loop optimal feed rate profile method. These problems are discussed in this section.

3.4.1 Singular control

The term singular control is used to describe a situation in which the optimal control equation (3-12) fails to determine an optimal control action or feed rate (F) (Noton, 1972; Ramirez, 1994; Teo, *et al.*, 1991). This can happen when the system equations and therefore the Hamiltonian are linear in the control variables. In general, if we assumed the absence of constraint on the control variable or feed rate, it can be shown that, during the singular period, the necessary condition for the optimality is satisfied (refer to Equation (3-12)):

$$\frac{\partial H}{\partial F} = \Psi = 0 \qquad (3\text{-}64)$$

However, the sufficient condition is not satisfied as:

$$\frac{\partial^2 H}{\partial F^2} = 0$$

This situation is probably one of the main reasons why the singular control is not preferable and several methods and transformations have been used to avoid it (Modak and Lim, 1989; Hong, 1986; Ohno, *et al.*, 1978).

Moreover, the singular control introduces further difficulties for determining the optimal feed rate profile. In the computational aspect, the difficulty arrives during the differentiation of Equation (3-64) until the feed rate (F) reappears. This algebraic manipulation can easily take several pages even for a simple fourth-order systems as

pointed out by Terwiesch, *et al.* (1994). Also, if numerical optimisation methods, particulary those based on a gradient search, are adopted, the convergence issue becomes eminent as the gradient ($\partial H/\partial F$) depends indirectly on the substrate feed rate (F).

3.4.2 Open loop control

The calculus of variations is used to determine optimal feed rate profiles that will optimise fed-batch fermentation processes. This results in a two-point boundary-value problem and because of the nonlinear nature of the processes, the optimal solution usually falls out as an open loop control algorithm. One advantage of this approach is that it does not need measurements of state variables which are often difficult to obtain on-line. Instead it assumes that the state variables are proceeding along known paths a-priori determined by models. However, the disadvantage of such an open loop approach is that the performance will severely deteriorate in the presence of process disturbances or plant-model mismatch.

3.5 Summary of the open loop optimal feed rate control method

The method for determining the optimal feed rate profile presented in this chapter is briefly summarised in this section. From the formulation of the Hamiltonian and costate equations, the optimal feed rate can be obtained by using the Pontryagin's maximum principle as there are constraints on the control variable. The optimal feed rate can be obtained from Equation (3-12), which is shown here:

$$\frac{\partial H}{\partial F} = -\frac{\lambda_X X}{V} + \lambda_V + \frac{\lambda_S (S_f - S)}{V} - \frac{\lambda_P P}{V} = \Psi \qquad (3\text{-}12)$$

The optimal feed rate is determined by the sign of Ψ as follows:

if $\Psi < 0$ then $F = 0$

if $\Psi > 0$ then $F = F_{max}$

if $\Psi = 0$ then $F = F_{sing}$

The fermentation process is started with the maximum or minimum feed rate depending on the sign of Ψ if the singular condition does not hold at the beginning of the batch. When the sign of Ψ becomes zero, which means the singular condition has been satisfied, the singular feed rate starts.

As the optimal feed rate profile comprises maximum, minimum and singular feed rates, the optimisation problem here is therefore reduced to the determination of the optimal feed rate sequence and the corresponding optimal switching time between these feed rates.

It is worth noting that the singular feed rate in Equation (3-32) and (3-62) for the primary and secondary metabolite production are model-based regulator control.

$$F_{sing} = \frac{\mu X V}{Y_{xs}\left(S_f - \sqrt{K_s K_i}\right)} \tag{3-32}$$

$$F_{sing} = \frac{V}{\left(S_f - S_{sing}\right)} \frac{\mu X}{Y_{xs}} \quad , \quad S_{sing} = S_{(d(\pi/\mu)/dS=0)} \tag{3-62}$$

The singular feed rate would control the substrate concentration constant at the level where the singular feed rate is started. The singular feed rate would continue until the constraints are reached either the singular feed rate becomes higher than the maximum feed rate or the culture volume reaches the maximum. At this point, the singular condition could not be held any longer and the sign on Ψ will determine whether the maximum feed rate (the singular feed rate reaches the maximum feed rate constraint) or the minimum feed rate (the reactor is full) is used. This also applies to the singular feed rate for the secondary metabolite production when the operating cost is considered (Equation (3-59)). However,

the substrate concentration follows the trajectory generated by Equation (3-58) and is not constant.

$$F_{\text{sing}} = \frac{V}{\left(S_f - S_{\text{sing}}\right)}\left(\frac{\mu X}{Y_{xs}} + \frac{\mu'\left(\pi'\mu - \pi\mu'\right)}{\left(\pi'\mu'' - \pi''\mu'\right)}\right) \tag{3-59}$$

It is obvious that insight into the phenomena occurring in the fed-batch fermentation processes under the optimal control can be gained from considering the substrate concentration in a bioreactor. It is the substrate feed rate which is manipulated by the sign of Ψ that governs the substrate concentration and resulting in desired product obtained. This emphasises the important of substrate concentration to optimise the fermentation processes. Also, it is not until recently that on-line estimation of state variables has been successfully developed and used in the industry (Zhang, *et al.*, 1996). Therefore in next chapter, we propose an optimisation in which the optimal substrate concentration is first determined and then the corresponding feed rate is calculated. The advantages of the proposed method will be shown in Chapter 5 in which these two methods are compared.

Chapter 4 Closed Loop Optimal Control of Fermentation Processes

4.0 Introduction

In the previous chapter, an optimal control for maximising a given objective function in fermentation processes has been analysed. It was shown that the objective function is optimised by using substrate feed rate to control substrate concentration following the optimal path. This clearly showed the effect of the substrate concentration to metabolite production in the fermentation processes. From the microbiological point of view, this result is not unexpected, as the specific reaction rates (specific growth rate (μ), specific substrate consumption rate (σ) and specific product formation rate (π)) in the fermenter are governed by the environmental conditions of the fermenter such as the substrate concentration. The purpose of the substrate feed rate is therefore to maintain the substrate concentration in the reactor at favourable levels.

The relationship between the substrate feed rate and substrate concentration is shown in a material balance equation (Equation (2-10)) of substrate concentration in Chapter 2 and is also illustrated in Figure 4-1 in which the substrate feed rate with concentration S_f is fed into the fermenter and diluted to concentration S.

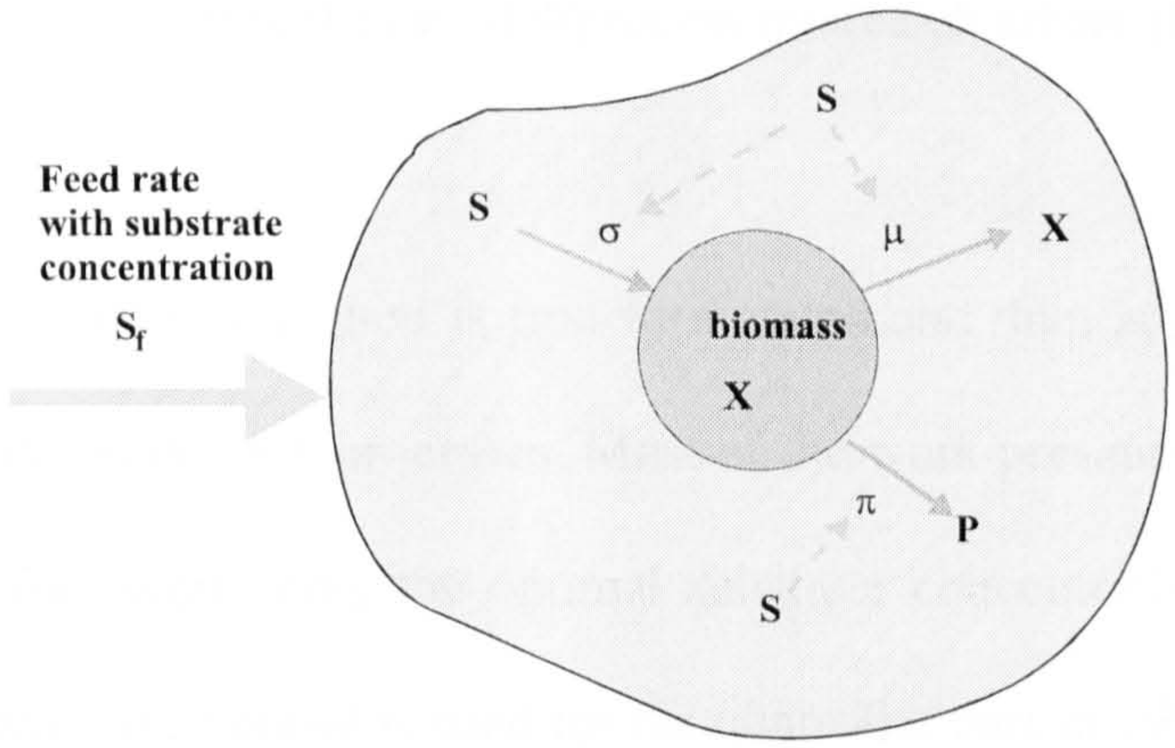


Figure 4-1 Diagram shows the reaction in the fermenter

The main task for optimisation of the fed-batch fermentation processes is thus to control the substrate concentration following the optimal trajectory in order to optimise the desired objective function. Hence, we propose a method, which separates the optimal control problem formulated in the previous chapter into two parts. The first part is to determine the optimal trajectory of the substrate concentration. The second part is to design a controller to track the obtained substrate concentration trajectory. It is also due to the recently development of on-line estimation of the substrate concentration (Zhang, *et al.*, 1996) that makes the proposed method applicable.

The advantages of this method over the optimal feed rate profile method are two fold. First, this method can avoid the singular problem, which usually occurs in the optimal feed rate profile method. This is because the substrate concentration appears nonlinearly in the system equations. Secondly, the controller is operated in a feedback mode for controlling the obtained substrate concentration trajectory. The closed loop control problem is preferable over the open loop one as external disturbances can be compensated. The proposed method also offers the flexibility that many types of controllers can be designed

and used particularly those robust to model/process mismatch errors (Green and Limebeer, 1994).

In this chapter, the proposed method is first formulated and then applied to primary and secondary metabolite production processes. Most of the work presenting in this chapter is, however, devoted for determining the optimal substrate concentration profile. Although the model based predictive control is used for the controller part in this thesis, it would not be in detail as other types of controller can also be exploited as long as they are capable of controlling the substrate concentration to follow the optimal profile.

4.1 Optimal Substrate Concentration Profile and Feedback Control

In this section, the optimal control problem formulated in the previous chapter is divided into two parts. The first part is to determine the optimal substrate concentration profile. This optimisation is also performed by the calculus of variations method, but here the control variable is changed from substrate feed rate to substrate concentration in the fermenter instead. A model predictive control is then used to control the obtained substrate concentration trajectory.

These two parts are complementary with each other. In designing the optimal substrate profile, both substrate feed rate and volume are omitted from the system model. The controller is then not only used to cope with the omitting of feed rate and volume constraints but also to control the substrate concentration following the optimal path. Since this provides a closed loop control of the substrate concentration that optimises the given objective function, this strategy is called "closed loop optimal control".

4.1.1 Optimal substrate profile determination

The calculus of variations method is used to determine an optimal substrate concentration profile that will optimise the objective function. Since the substrate feed rate and volume are omitted, the system equations become:

$$\frac{dX}{dt} = \mu X \tag{4-1}$$

$$\frac{dP}{dt} = \pi X \tag{4-2}$$

The objective function to be maximise is a function of biomass and/or product concentration at the final operating time as:

$$J(S) = f\left(X(t_f), P(t_f)\right) \tag{4-3}$$

The Hamiltonian and costate equations can then be written as:

$$H = \lambda_X \mu X + \lambda_P \pi X \tag{4-4}$$

$$\dot{\lambda}_X = -\frac{\partial H}{\partial X} = \lambda_X \mu + \lambda_P \pi \tag{4-5}$$

$$\dot{\lambda}_P = -\frac{\partial H}{\partial P} = 0 \tag{4-6}$$

Transversality conditions:

$$\lambda_X(t_f) = \frac{\partial J}{\partial X_{tf}}$$

$$\lambda_P(t_f) = \frac{\partial J}{\partial P_{tf}}$$

Where X_{tf} and P_{tf} are biomass and product concentration at the final time.

$$X_{tf} = X(t_f)$$

$$P_{tf} = P(t_f)$$

Optimal control:

$$\frac{\partial H}{\partial S} = \lambda_x X \frac{\partial \mu}{\partial S} + \lambda_P X \frac{\partial \pi}{\partial S} = 0 \qquad (4\text{-}7)$$

The optimal substrate concentration profile (S_{opt}) can be obtained by solving equation (4-7) for the substrate concentration (S). Note also that the optimal control here has no singular period due to the fact that the control variable, which is the substrate concentration (S) in this case, appears nonlinearly in the system and the Hamiltonian equations. (The specific reaction rates (μ, π) are nonlinear functions of substrate concentration.)

4.1.2 Controller design

In this subsection, a controller is designed to control the substrate concentration following the optimal profile. It has been mentioned earlier that one of the flexibilities of this method is that many types of controller can be used to control the obtained optimal substrate profile. However, a model based control scheme is used here so that the similarity of both the open loop optimal feed rate profile and the proposed closed loop control method can be maintained as much as possible, and the comparison of both methods in Chapter 5 is easily undertaken. Moreover, the model predictive control scheme and the optimal control scheme bear a very similar aspect as both employ the optimisation method to calculate the input variable. The main difference might be the implementation of the receding horizon by the model based control scheme and hence benefit the feedback advantage. The open loop optimal feed rate profile uses a process model for generating the optimal feed rate to

optimise the given objective function (It was shown in Chapter 3 that the optimal substrate feed rate is used to control the substrate concentration at the favourable condition for the product formation). The proposed method should also yield the similar result, since it uses the same process model to generate the optimal substrate concentration profile in the first step and the substrate feed rate to follow the desired substrate profile in the second step. Therefore the comparison of both methods should be justified.

Another reason for using the model based control is that the calculus of variations method has already required the process model for determining the optimal substrate profile. We therefore make use of the process model in designing a controller. Since the process model is nonlinear, the nonlinear model predictive control is used here for the tracking component of the proposed method. (Further details on nonlinear model predictive control are given in (Ali and Zafiriou, 1993; Biegler, 1991; Cuthrell and Biegler, 1987; Eaton and Rawlings, 1990; Renfro, et al., 1987; Sistu and Bequette, 1991).)

Nonlinear model predictive control belongs to a class of model predictive control, in which process model is used explicitly to determine a control law. Model predictive control is defined (Biegler, 1991; Eaton and Rawlings, 1992) as "a control scheme in which the controller determines a manipulated variable profile that optimises some open loop performance objective on a time interval extending from the current time to the current time plus a prediction horizon". Feedback is incorporated when a further measurement becomes available and the optimisation procedures for determining the manipulated variable profile is then restarted. This characteristic is called a receding horizon or finite moving horizon approach. The general problem to be solved by model predictive control can be stated as:

$$\min_{u(t)} \quad \Phi[u(t), x(t), y(t)]$$

subject to the system equations, constraints and initial conditions:

$$\frac{dx}{dt} - f(x,u) = 0$$

$$y - g(x,u) = 0$$

$$h(x,u) = 0$$

$$k(x,u) \geq 0$$

$$x(t_0) = x_0$$

with

$$t \in [t_0, t_0 + T]$$

The notation here refers only to this subsection: u is the input vector, y is the output vector and x is the state vector. The time interval is from the current time t_0 to $t_0 + T$, in which T is the length of the prediction horizon. The functional Φ is the performance objective of the controller. The functions *f* and *g* represent the process model, and *h* and *k* are equality and inequality constraints. Model predictive control is therefore suitable for the proposed scheme since the constraints on volume and feed rate can be handled explicitly.

The nonlinear model predictive control applied to a fed-batch fermentation process under the proposed control scheme can then be stated as follows where the substrate feed rate (F), which is the control variable can be obtained.

$$\min_{F(k)\ldots F(k+m-1)} \quad \sum_{i=1}^{p} \left(\hat{S}(k+i/k) - S_{opt}(k+i) \right)^2$$

where

$\hat{S}(k+i/k)$: model predicted value of substrate concentration at time (k+i) based on information at time k.

$S_{opt}(k+i)$: optimal substrate concentration at time (k+i).

p : prediction horizon

m : control horizon: ($F(k+i)=0 \quad \forall i \geq m \quad ; \quad m<p$)

subject to

$$\frac{dX}{dt} = \mu X - DX \quad ; \quad X(0) = X_0$$

$$\frac{dS}{dt} = -\frac{1}{Y_{xs}}\mu X + D(S_f - S) \quad ; \quad S(0) = S_0$$

$$\frac{dP}{dt} = \pi X - DP \quad ; \quad P(0) = P_0$$

$$\frac{dV}{dt} = F \quad ; \quad V(0) = V_0$$

$$0 \leq F \leq F_{\max}$$

$$V(t_f) \leq V_f$$

with

$$t \in [t_0, t_0 + T]$$

4.2 Closed Loop Optimal Control of Primary and Secondary Metabolite Production

The proposed closed loop optimal control method is applied to primary and secondary metabolite production processes in this section. Note that most part of the work presented here will refer to only the determination of substrate concentration profile. The controller will be included and used in the next chapter.

4.2.1 Primary metabolite production

A biomass production process is used as the primary metabolite fermentation process. The process model is therefore written as:

$$\frac{dX}{dt} = \mu(S)\ X \tag{4-8}$$

The specific growth rate (μ) is a function of substrate concentration (S), which is used as the control variable. The aim is to maximise the biomass concentration at the final operating time, which can be transformed into the objective function as:

$$J(S) = X(t_f) - \varepsilon \int_{t_0}^{t_f} dt \tag{4-9}$$

Where ε is the cost factor per unit of operating time. The Hamiltonian and costate equations are then written as:

$$H = -\varepsilon + \lambda_x \mu X \tag{4-10}$$

$$\dot{\lambda}_x = -\frac{\partial H}{\partial X} = -\lambda_x \mu \tag{4-11}$$

Transversality condition:

$$\lambda_x(t_f) = \frac{\partial J}{\partial X_{tf}} = 1$$

The optimal control or the optimal substrate concentration profile in this case will be calculated from:

$$\frac{\partial H}{\partial S} = \lambda_x X \frac{\partial \mu}{\partial S} = 0 \qquad (4\text{-}12)$$

which implies that,

$$\frac{\partial \mu}{\partial S} = 0 \qquad (4\text{-}13)$$

This means that the optimal substrate concentration is the value that maximise the specific growth rate. This also coincides with the intuition that to maximise the biomass concentration, the specific growth rate needs to be maximised. If we recall the optimal control of biomass production in the previous chapter, it can be seen that the condition in Equation (4-13) is the condition that singular feed rate occurs.

Two common structures of the specific growth rate (μ) are also examined here. The first is the Monod typed kinetic and the second is the substrate inhibition typed kinetic.

4.2.1.1 Monod kinetic

The relationship between specific growth rate (μ) and substrate concentration (S) for the Monod kinetic is shown in Equation (4-14) and can be seen in Figure 2-5 in Chapter 2.

$$\mu = \frac{\mu_{max} \cdot S}{K_s + S} \qquad (4\text{-}14)$$

It can be seen, as in Section 3.2.1.1, that the specific growth rate would be maximised when the substrate concentration is kept as high as possible. This knowledge makes the

biomass production with this kinetic operates in batch mode. (The fermenter is filled up at the beginning of the batch to keep the substrate concentration at high level. The process is then started in batch mode.)

4.2.1.2 Substrate inhibition kinetic

The relationship between specific growth rate (μ) and substrate concentration (S) for the substrate inhibition kinetic is shown in Equation (4-15) and can be seen in Figure 2-6 in Chapter 2.

$$\mu = \frac{\mu_{max} \cdot S}{K_s + S + S^2/K_i} \qquad (4\text{-}15)$$

It can be shown that specific growth rate would be maximised when the substrate concentration is kept at the following level, which is determined from Equation (4-13):

$$S_{opt} = \sqrt{K_s \cdot K_i} \qquad (4\text{-}16)$$

The biomass production in case of the substrate inhibition kinetic is therefore operated in fed-batch mode in order to keep the substrate concentration constant at the optimal level (S_{opt}), which maximises the specific growth rate.

4.2.2 Secondary metabolite production

The process model for a secondary metabolite production can be written as:

$$\frac{dX}{dt} = \mu(S)\ X$$

$$\frac{dP}{dt} = \pi(S)\ X$$

The specific growth rate (μ) and the specific product formation rate (π) are functions of the substrate concentration (S) which is the control variable. The aim of this process is to maximise the secondary metabolite product (P) at the final operating time. Therefore the objective function can be written as:

$$J(S) = P(t_f) - \varepsilon \int_{t_0}^{t_f} dt \tag{4-17}$$

Where ε is the cost factor per unit of operating time. The Hamiltonian and costate equations can then be written as:

$$H = -\varepsilon + \lambda_X \mu X + \lambda_P \pi X \tag{4-18}$$

$$\dot{\lambda}_X = -\frac{\partial H}{\partial X} = -\lambda_X \mu - \lambda_P \pi \tag{4-19}$$

$$\dot{\lambda}_P = -\frac{\partial H}{\partial P} = 0 \tag{4-20}$$

Transversality conditions:

$$\lambda_X(t_f) = \frac{\partial J}{\partial X_{tf}} = 0 \tag{4-21}$$

$$\lambda_P(t_f) = \frac{\partial J}{\partial P_{tf}} = 1 \tag{4-22}$$

The optimal control or optimal substrate concentration profile can be determined from:

$$\frac{\partial H}{\partial S} = \lambda_X X \frac{\partial \mu}{\partial S} + \lambda_P X \frac{\partial \pi}{\partial S} = 0 \tag{4-23}$$

Since the biomass concentration (X) is not zero, this implies that,

$$\lambda_X \frac{\partial \mu}{\partial S} + \lambda_P \frac{\partial \pi}{\partial S} = 0 \tag{4-24}$$

As the Hamiltonian (H) is zero for a free time problem (the final operating time (t_f) is not fixed), the Hamiltonian equation (4-18) becomes:

$$H = -\varepsilon + \lambda_X \mu X + \lambda_P \pi X = 0$$

or

$$X = \frac{\varepsilon}{\lambda_X \mu + \lambda_P \pi} \tag{4-25}$$

Find the value of λ_X in Equation (4-24) and substitute into Equation (4-25) yields,

$$X = -\frac{\mu' \varepsilon}{(\pi' \mu - \pi \mu')} \tag{4-26}$$

where $'$ is the differentiation with respect to the substrate concentration (S). Equation (4-26) shows a relationship between the biomass and substrate concentration at the different cost factors (ε).

Taking the time derivative of Equation (4-24) yields,

$$-\lambda_X \mu \mu' - \lambda_P \pi \mu' + \lambda_X \mu'' \dot{S} + \lambda_P \pi'' \dot{S} = 0$$

And substitute with λ_X from (4-24) and λ_P from (4-20) and (4-22) results in:

$$\dot{S} = -\frac{\mu' (\pi' \mu - \pi \mu')}{(\mu' \pi'' - \pi' \mu'')} \tag{4-27}$$

Equation (4-27) represents the optimal substrate concentration profile that maximises the given objective function (4-17). Note that there is no biomass concentration in this equation. The relationship between substrate and biomass concentration has been however shown earlier in Equation (4-26). During the process optimisation, these two equations suggest that the substrate concentration needs to be controlled following the optimal profile in Equation (4-27), while the relationship between the biomass and substrate

concentration in Equation (4-26) is also hold. This illustrates the fact that the production of secondary metabolite depends not only on substrate concentration (suitable condition of microbial production) but also on biomass (sufficient amount of biomass for microbial production).

In another case, where the objective function does not take the operating cost into account, the objective function is written as:

$$J(S) = P(t_f)$$

Following the same procedure, The optimal control or optimal substrate concentration can be determined from:

$$\frac{\partial H}{\partial S} = \lambda_X X \frac{\partial \mu}{\partial S} + \lambda_P X \frac{\partial \pi}{\partial S} = 0 \qquad (4\text{-}28)$$

or

$$\lambda_X \frac{\partial \mu}{\partial S} + \lambda_P \frac{\partial \pi}{\partial S} = 0 \qquad (4\text{-}29)$$

For a free final time case, the Hamiltonian (H) equals zero and equation (4-18) in which ε is zero becomes:

$$\lambda_X \mu + \lambda_P \pi = 0 \qquad (4\text{-}30)$$

Find the value of λ_X in (4-30) and substitute into (4-29) yields,

$$\lambda_P (\pi \mu' - \mu \pi') = 0$$

From Equation (4-20) and (4-22), λ_P is not zero. This implies that,

$$(\pi \mu' - \mu \pi') = 0$$

or

$$\frac{d(\pi/\mu)}{dS} = 0 \tag{4-31}$$

This means that the optimal substrate concentration is to be kept at the level which maximises the ratio between the specific product formation rate and the specific growth rate. Note that equation (4-31) is the same condition that occurs during the singular period in the previous chapter (refer to equation (3-63)). The same explanation can also be used.

4.3 Substrate Feed Rate Profile and Optimal Substrate Concentration Profile

In this section, the pattern of feed rate that would result in the optimal substrate profile is discussed and compared with those from the open loop optimal feed rate control in the previous chapter.

As the sign of Ψ in the open loop optimal feed rate control method in the previous chapter that designs the desired feed rate as minimum, maximum or singular, it is the optimal substrate concentration profile and the constraints on feed rate and culture volume that determine the corresponding feed rate in the proposed method. This can be seen, for example, if the initial substrate concentration is higher or lower than the optimal substrate concentration, the response from the controller is to drive the substrate concentration to the desired substrate profile as fast as possible therefore resulting in the minimum or maximum feed rate as bounded by the feed rate constraints. The same result is also applied when the reactor is full and the controller responds with the minimum feed rate. During the controller tracking the optimal substrate concentration profile, the constraints on feed rate might be activated again when the substrate concentration need higher feed rate than

the maximum level to maintain the substrate concentration following the optimal profile. This also results in the maximum feed rate while the substrate concentration starts deviate from the optimal path due to constraints.

In Chapter 3, we start from the optimal feed rate profile and lead to the condition of optimal substrate profile. However, in this chapter, the process is reverse and we would start from the optimal substrate profile and then envisage the pattern of the optimal feed rate. This analysis also gives an insight look in the optimisation of fed-batch fermentation processes particularly the similar and difference between the method in Chapter 3 and the one in this Chapter. This is also illustrated in Figure 4-1, where the upper part refers to the open loop optimal control and the lower part refers to the closed loop optimal control. In the open loop optimal control, the optimal substrate feed rate profile is applied to the process, which results in the maximum product being obtained. In the closed loop optimal control, the optimal substrate concentration profile is directly determined to maximise the objective function. The substrate feed rate is then later calculated to provide this optimal substrate concentration profile.

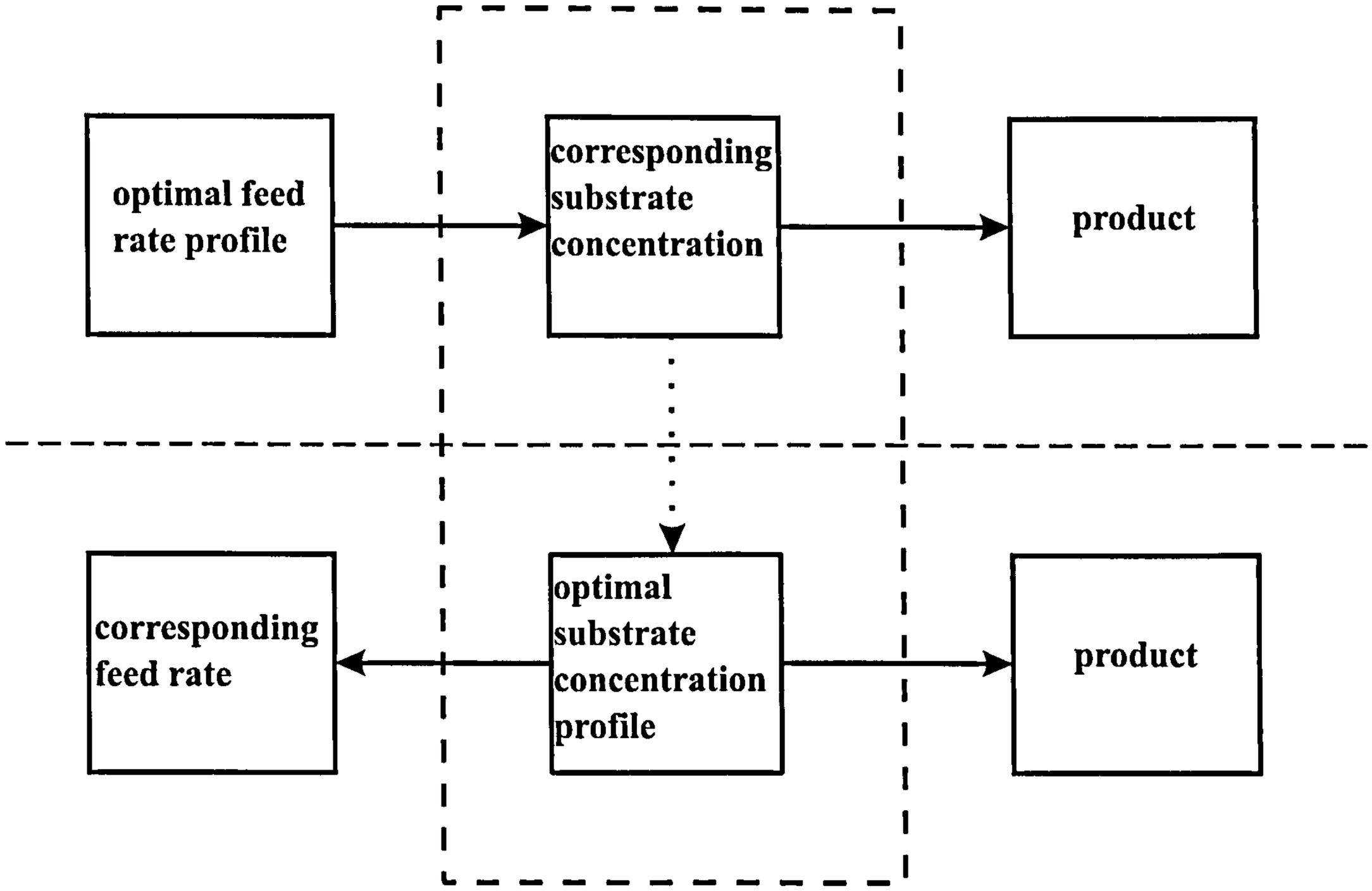


Figure 4-2 Diagram shows the relation between optimal feed rate profile and optimal substrate profile

Since the specific reaction rates are functions of substrate concentration in this thesis, it is also worth mentioning that when the specific reaction rates (μ, σ and π) are functions of other environmental variables such as pH, temperature, etc. This method can still be used by determining the trajectories of these variables instead of substrate concentration.

4.4 Summary of the closed loop optimal control method

The proposed method of closed loop optimal control is summarised in this section. As the analysis showed the importance of substrate concentration on the bioreaction rates, the closed loop optimal control divides the general optimal control problem into two parts as:

1. determine an optimal substrate concentration profile

2. design a controller to track the obtained optimal substrate concentration profile

The optimal substrate concentration profile is determined using the calculus of variations method, which is shown in Equation (4-7):

$$\frac{\partial H}{\partial S} = \lambda_x X \frac{\partial \mu}{\partial S} + \lambda_p X \frac{\partial \pi}{\partial S} = 0 \qquad (4\text{-}7)$$

The optimal substrate concentration profile is determined without considering the effect of constraints on feed rate and culture volume. Therefore it is actually an ideal optimal path for the optimisation of the fermentation process. The omitted constraints are accommodated in the second part of the method in which the nonlinear model predictive control is designed for tracking the optimal substrate concentration profile.

Chapter 5 Relationship and Comparison between Open Loop and Closed Loop Optimal Control.

5.0 Introduction

Optimisation of fed-batch fermentation processes by the calculus of variations or Lagrange optimisation has been presented in Chapter 3. It resulted in an open loop optimal control that consisted of a time sequence of maximum, minimum and singular feed rates. It was found that the singular feed rate was used to control the substrate concentration at the optimal level. This has led to the proposed method in Chapter 4, in which the optimal control problem is divided into determining an optimal substrate concentration profile and controller design. The proposed method not only avoids the singular control problem, which occurs in the system that is linear in control variables, but also operates the system in closed loop. Since the substrate concentration is controlled in closed loop mode following the optimal trajectory, which satisfies the objective function, this strategy is therefore called "closed loop optimal control" as mentioned in the previous chapter.

In this chapter, we use results from Chapter 3 and Chapter 4 to establish the relationship between each other. Performances of both methods are also compared. This is done by performing simulation of primary and secondary metabolite production processes. The comparison will also applied to both perfect process model and plant/model mismatch cases.

5.1 Relationship between Open Loop and Closed Loop Optimal control

It has been shown in Chapter 3 and Chapter 4 that the open loop optimal feed rate profile was intended to maintain the substrate concentration at the optimal level or trajectory. These results are similar for both primary and secondary metabolite production processes. However, the closed loop optimal control is more transparent and flexible. Any controller can be used to achieve an objective of keeping substrate concentration at the optimum level or profile (S_{opt}), which is known to maximise the biomass or product production. This allows for additional features such as closed loop robustness or disturbance rejection capabilities to be integrated. Comparing with the method for calculating open loop optimal feed rate profile, feed rate sequence is pre-determined to maximise metabolite production in one step. Therefore, the optimal substrate concentration level is not explicitly shown and transparent to the operator. The closed loop optimal control also gives the closed loop control while the open loop optimal feed rate method results in open loop control. Block diagrams comparing both methods are shown in Figure 5-1 and Figure 5-2.

Moreover, the proposed method gives the optimal profile which is specific to the process and micro-organism strains. In this case, substrate concentration is to be kept at S_{opt}. The optimal substrate concentration profile (S_{opt}) is independent of constraints on minimum and maximum feed rates. This provides more flexibility in its usage comparing to the open loop optimal feed rate profile method in which changing in minimum or maximum feed rate due to physical process constraints would result in the different time length in the combination of each feed rates and therefore different feed rate profiles. Table 5-1 summarises the comparison between both methods.

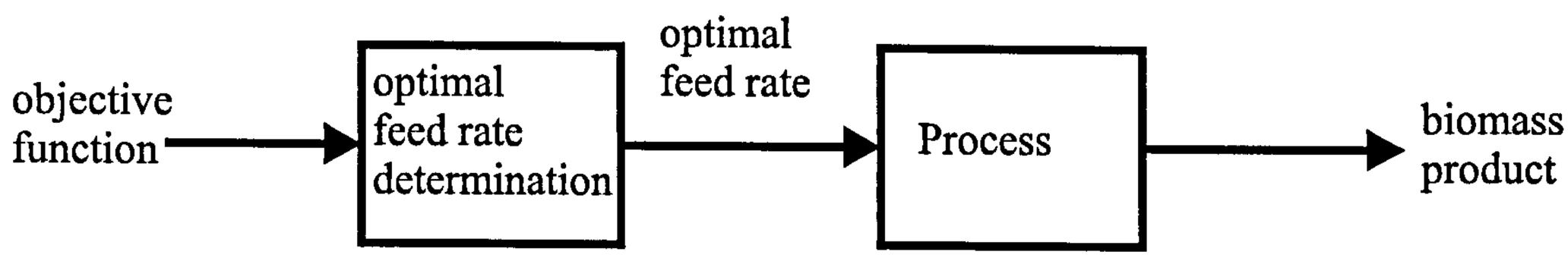


Figure 5-1 Block diagram for open loop optimal feed rate profile method.

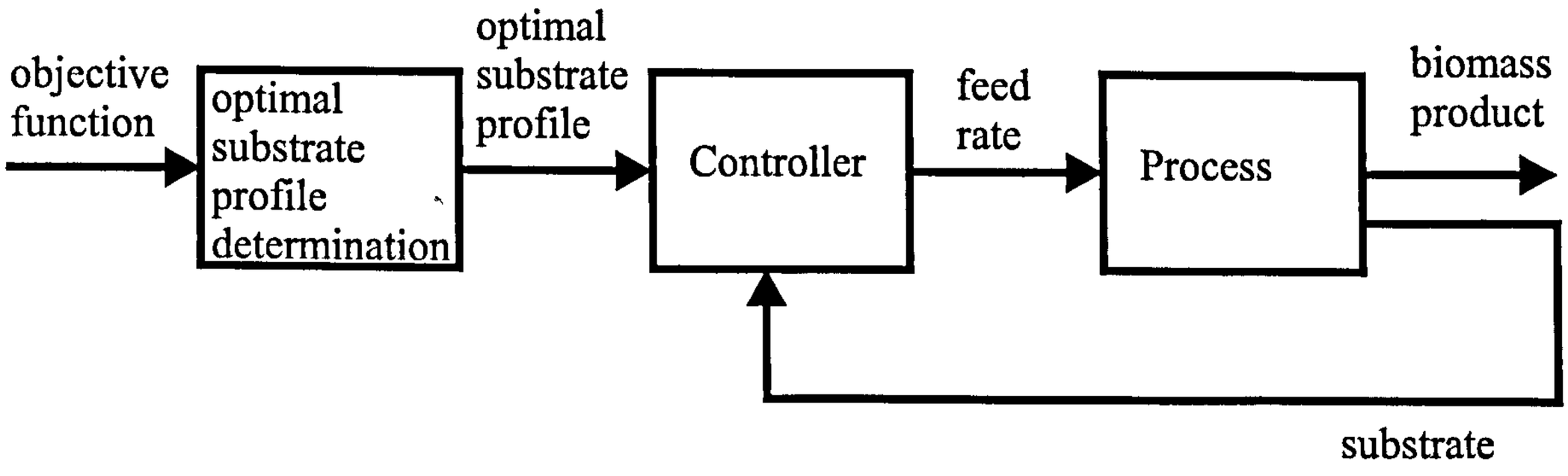


Figure 5-2 Block diagram for closed loop optimal control method.

Table 5-1 Summary of the comparison between both methods

No.	Open Loop Optimal Feed Rate Profile	Closed Loop Optimal Control
1	switching time depends on feed rate constraints (e.g., change in minimum or maximum feed rate results in different switching time)	Controller objective is to track the optimal substrate profile, which is independent of the minimum and maximum feed rate constraints. optimal substrate concentration profile is identical for the same process operating at different feed rate constraints.
2	problem of singular control due to feed rate is linear in the Hamiltonian	no problem of singular control
3	difficult to understand and not transparent	easy to understand and transparent.
4	feed rate has direct effect to an objective function (optimal feed rate is determined to satisfy the objective function.)	substrate concentration has direct effect to an objective function (optimal substrate concentration is determined and then feed rate is used to control the substrate concentration following the optimal profile.)
5	off-line pre-calculation of feed rate	on-line feedback control of substrate concentration

In next section, the performance of both methods are compared by simulation performing on primary and secondary metabolite production processes.

5.2 Performance Comparison

In this section, performance of the closed loop optimal control (CLOC) using primary and secondary metabolite production processes is demonstrated and compared with the open loop optimal feed rate profile method (OLOFP). For the primary metabolite production, a biomass production process is used as an example.

In both processes, the substrate inhibition kinetic is employed as it can give the finite optimal level and suitable for comparison. Moreover, it is the this type of kinetic that can be used to model the catabolite repression effect and thus requiring the fermentation process to operate in fed-batch mode. (refer to Section 3.2.1.1 and 3.2.1.2 in Chapter 3 for discussion on the Monod and substrate inhibition kinetic.)

5.2.1 Primary metabolite production (Biomass production process)

The fed-batch fermentation models are those shown in Chapter 2 (refer to equation (2-24) to (2-27)) and are written here as:

$$\frac{dX}{dt} = \mu X - DX \tag{5-1}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{xs}}\mu X + D(S_f - S) \tag{5-2}$$

$$\frac{dV}{dt} = F \tag{5-3}$$

$$D = \frac{F}{V} \tag{5-4}$$

where the kinetic reaction of the specific growth rate is in the substrate inhibition form:

$$\mu = \frac{\mu_{max} S}{K_s + S + S^2/K_i} \qquad (5\text{-}5)$$

The relationship between the specific growth rate (μ) and substrate concentration (S) is shown in Figure 5-3. The parameter values used for the simulation are in Table 5-2. Note that the parameters used for simulation are chosen arbitrary without any intention to be specific for a particular process but for representing a general characteristic for a class of processes with the substrate inhibition type kinetic.

Table 5-2 Parameters used in the simulation for a primary metabolite production

Parameter	Value	Unit
μ_{max}	0.10	(g biomass / (g biomass * hr))
K_s	3.0	(g substrate / litre)
K_i	8.34	(g substrate / litre)
Y_{xs}	0.164	(g biomass / g substrate)
X(0)	1	(g biomass / litre)
S(0)	20	(g substrate / litre)
V(0)	20	(litre)
V(tf)	50	(litre)
S_f	100	(g substrate /litre)

where

μ_{max} is the maximum specific growth rate

K_s and K_i are rate constant

Y_{xs} is yield of biomass from substrate

X(0), S(0) and V(0) are initial condition of biomass and substrate concentration, and culture volume

$V(t_f)$ is final culture volume

S_f is substrate concentration in the substrate feed stream

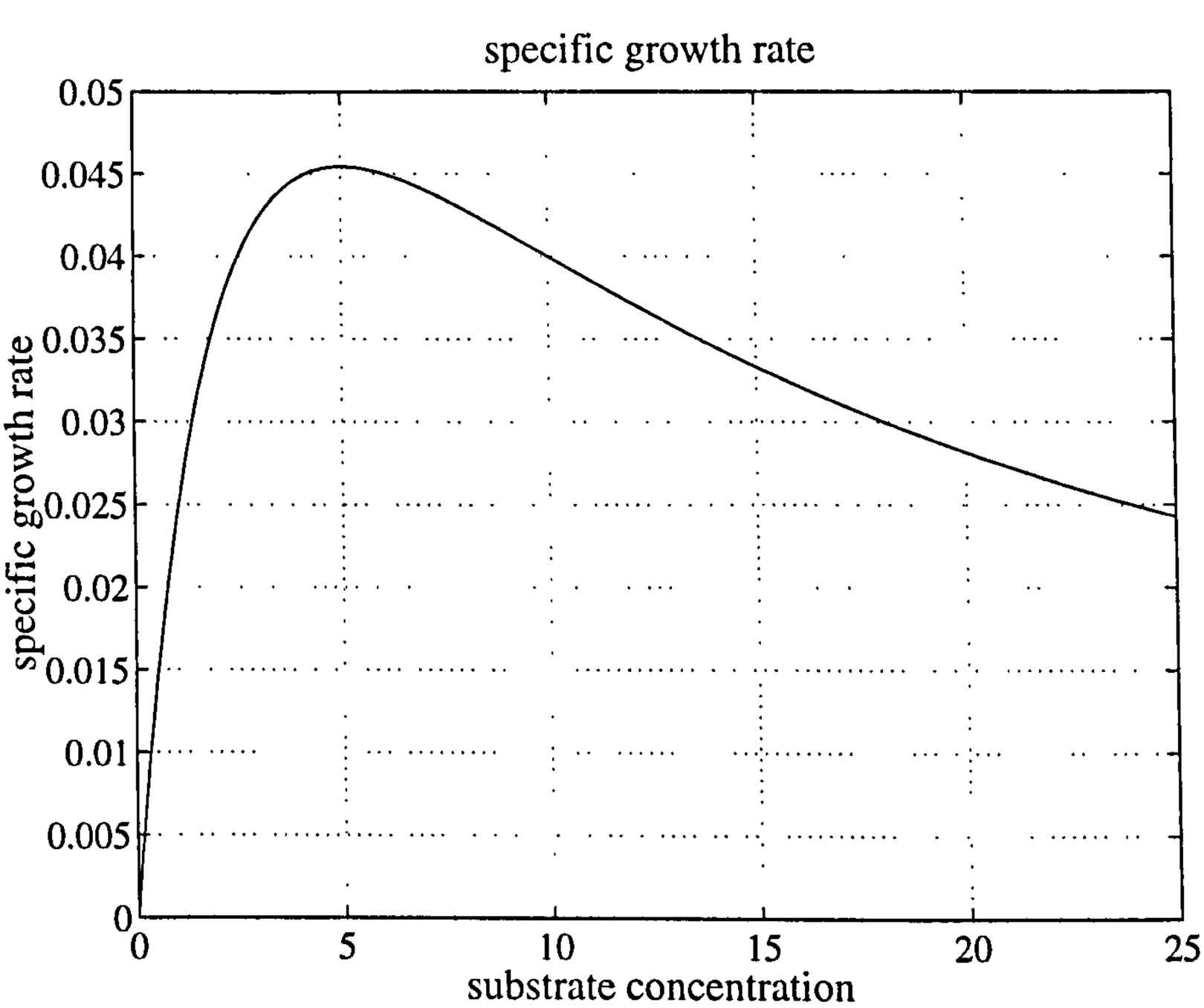


Figure 5-3 Relationship between the specific growth rate and substrate concentration

For OLOFP case, the optimal feed rate can be obtained from Equation 3-23,

$$\frac{\partial H}{\partial F} = -\frac{\lambda_x X}{V} + \lambda_v + \frac{\lambda_s (S_f - S)}{V} = \Psi \qquad (3\text{-}23)$$

where the optimal feed rate is determined from the following conditions:

if $\Psi < 0$ then $F = 0$

if $\Psi > 0$ then $F = F_{max}$

if $\Psi = 0$ then $F = F_{sing}$

The singular feed rate (F_{sing}) can be obtained from Equation (3-29) as:

$$F_{sing} = \frac{\mu X V}{Y_{xs}(S_f - S)} \qquad (5\text{-}6)$$

During the singular period, the substrate concentration is kept constant (refer to Equation 3-25). This singular substrate concentration (S_{sing}) can be obtained from Equation (3-31) as:

$$S_{sing} = \sqrt{K_s K_i} = 5$$

For the CLOC method, the optimal substrate concentration can be obtained from Equation (4-16) as:

$$S_{opt} = \sqrt{K_s K_i} = 5 \qquad (4\text{-}16)$$

In simulation of the CLOC method, the feasible parameters are chosen for using in the nonlinear model predictive controller as follows:

sampling time - 1 hr.

prediction horizon - 5 hr.

control horizon - 3 hr.

The comparison simulations will be performed in both perfect model and plant/model mismatch cases.

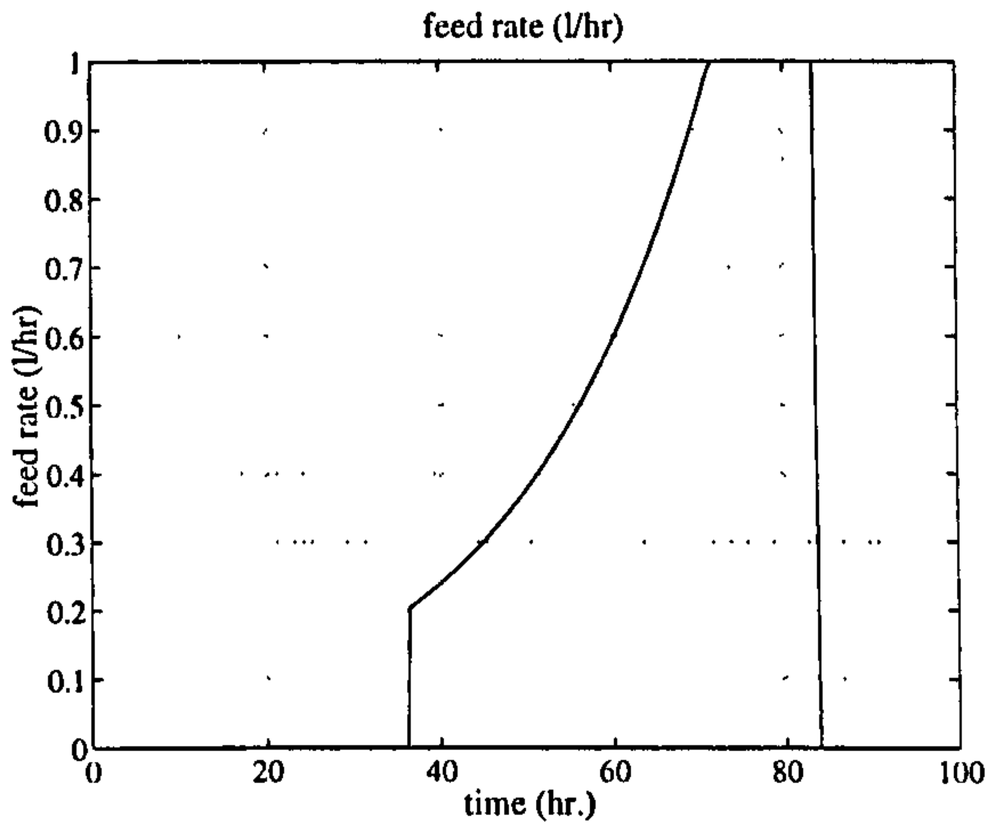
5.2.1.1 Control simulation with perfect model

Simulation results for the OLOFP and CLOC methods are shown in Figure 5-4 and Figure 5-5 respectively. (In each Figure 5-4 to Figure 5-11, (a) indicates feed rate, (b) substrate concentration, (c) biomass concentration and (d) culture volume.) It can be seen that in this case both methods give quite similar results. The constraints on minimum and maximum feed rate, which were omitted in the first part of the CLOC method, are well accommodated as shown in the simulation.

The optimal substrate concentration in this process is equal to 5 g/l. For the OLOFP case, it starts with minimum feed rate since the initial condition is higher than the singular level. Then the singular feed starts when the substrate level reaches the value of 5 g/l. The feeding continues to maintain substrate at this value until it is saturated. Finally the feed stops when the reactor is full. The same phenomena also appears in the CLOC case, where the controller tries to maintain the substrate concentration at 5 g/l. In the beginning of the process, since the substrate concentration is higher than the optimal level, therefore no substrate is fed into the process. The substrate level then decreases until it reaches the value of 5 g/l, when the controller starts feeding substrate into the process. Feed rate continues to maintain the substrate concentration in the fermenter at the optimal level (5g/l) until it is saturated and stops when the reactor is full. Although both methods give similar control action and results, it is mechanisms inside which are different. In next

subsection, an advantage of the CLOC method is demonstrated for the plant/model mismatch case.

In summary, both OLOFP and CLOC methods provide similar performance for the primary metabolite process under a perfect model situation.



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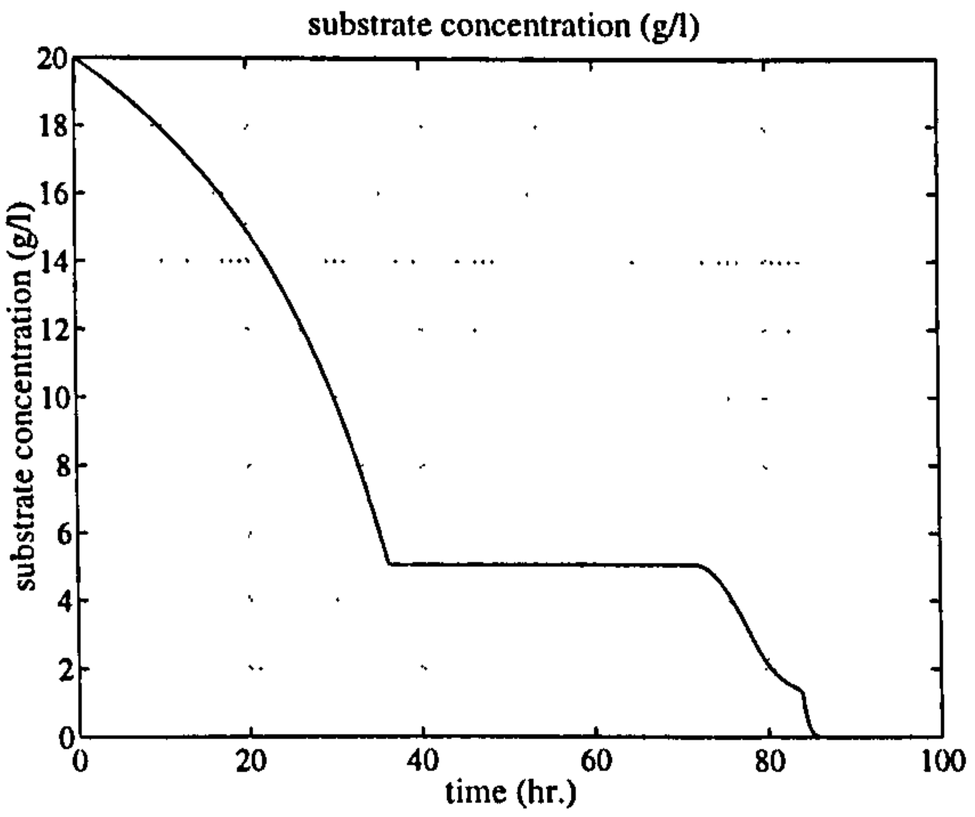

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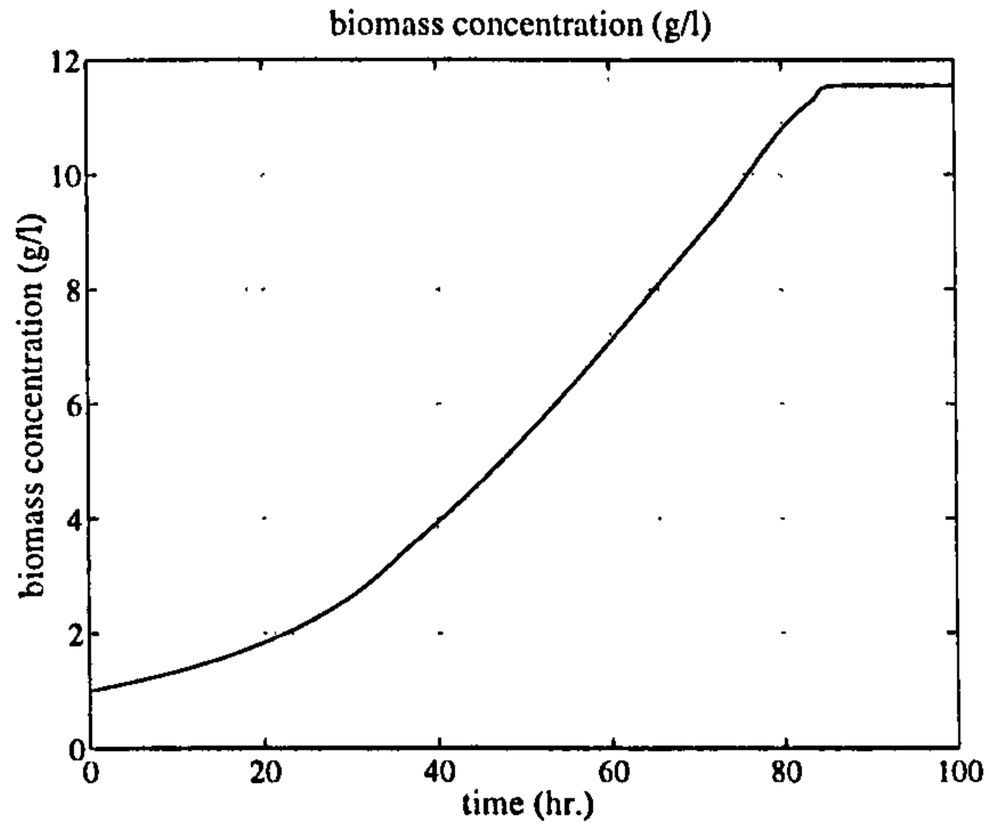

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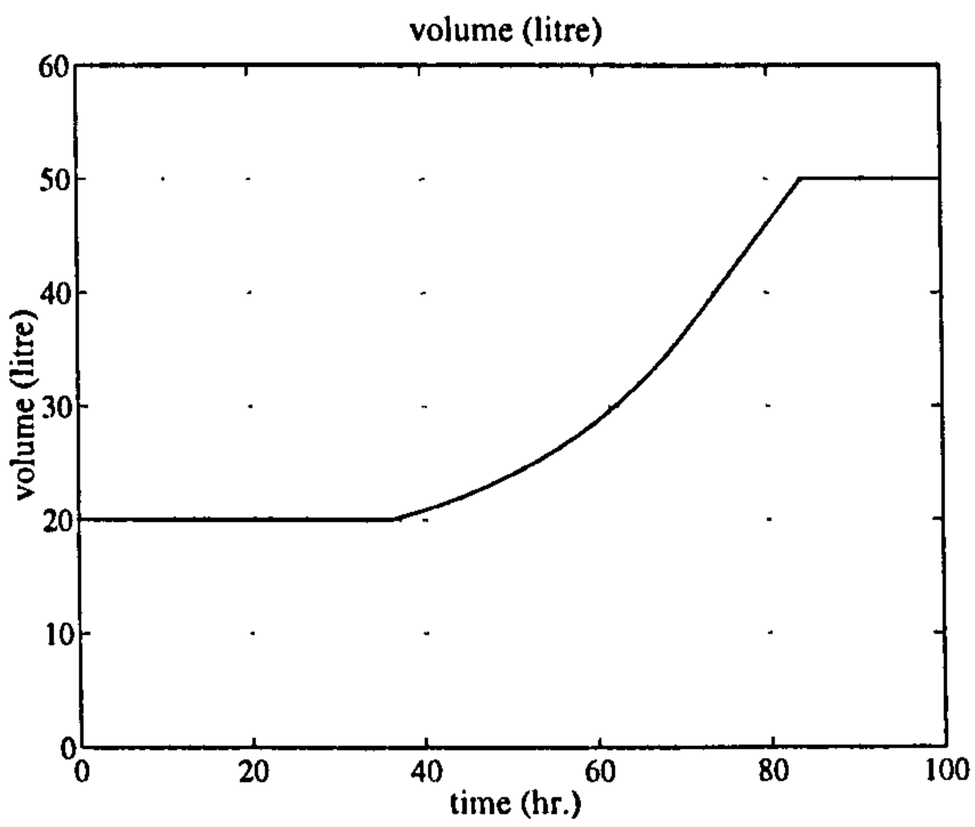
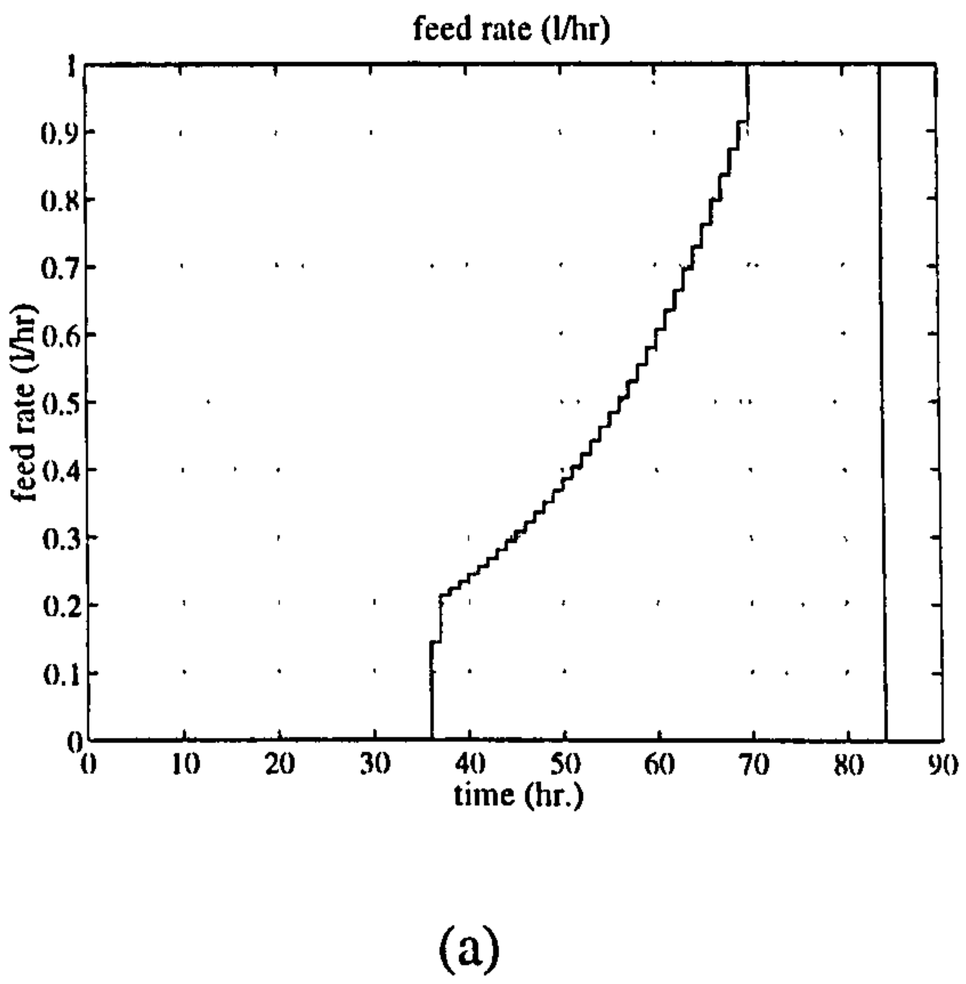


(d)

Figure 5-4 Results of a primary metabolite production for OLOFP (in perfect model)



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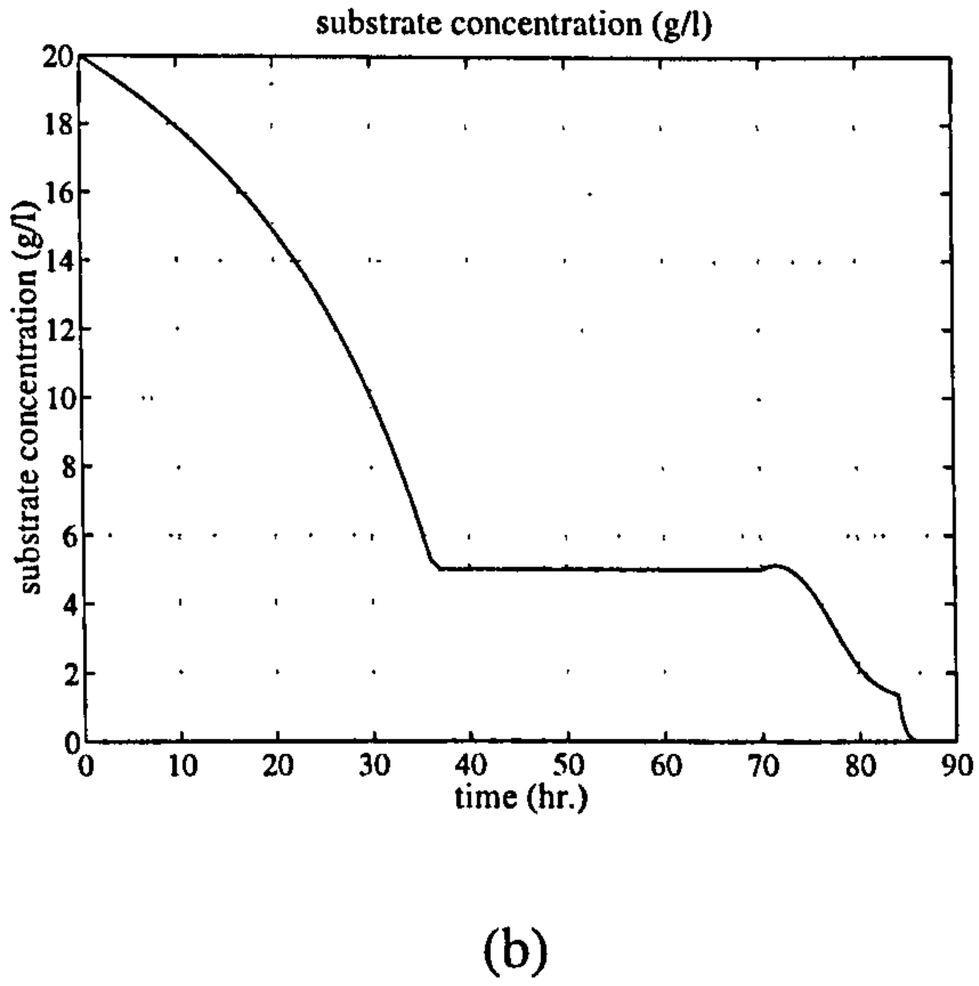

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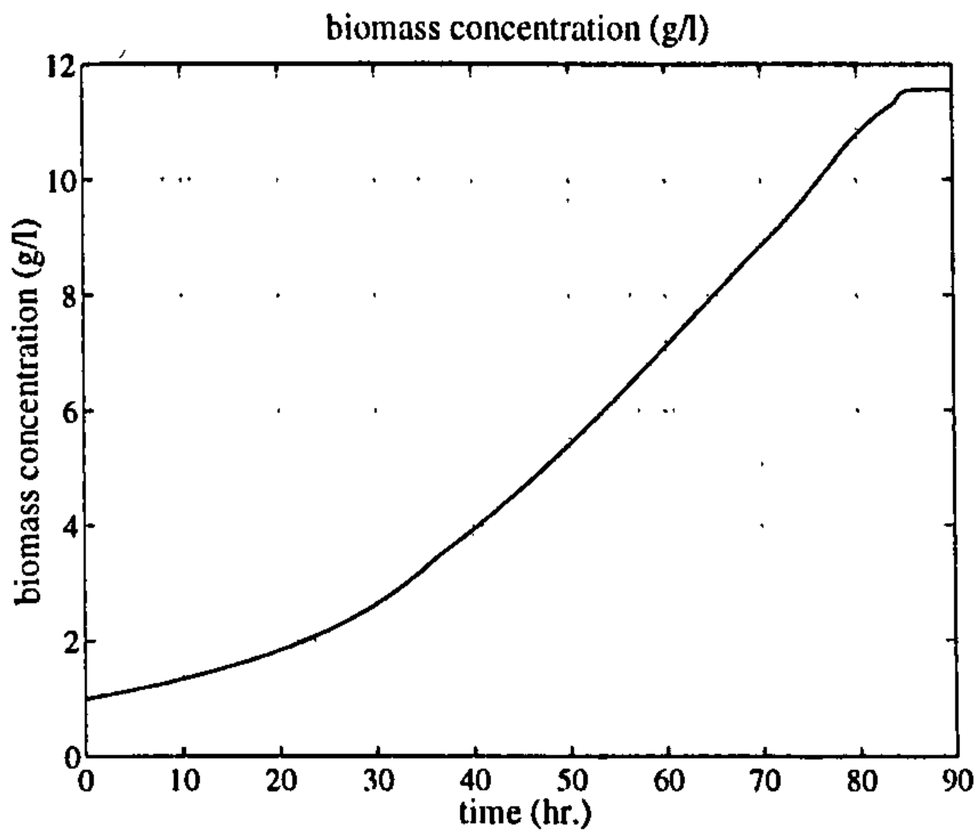

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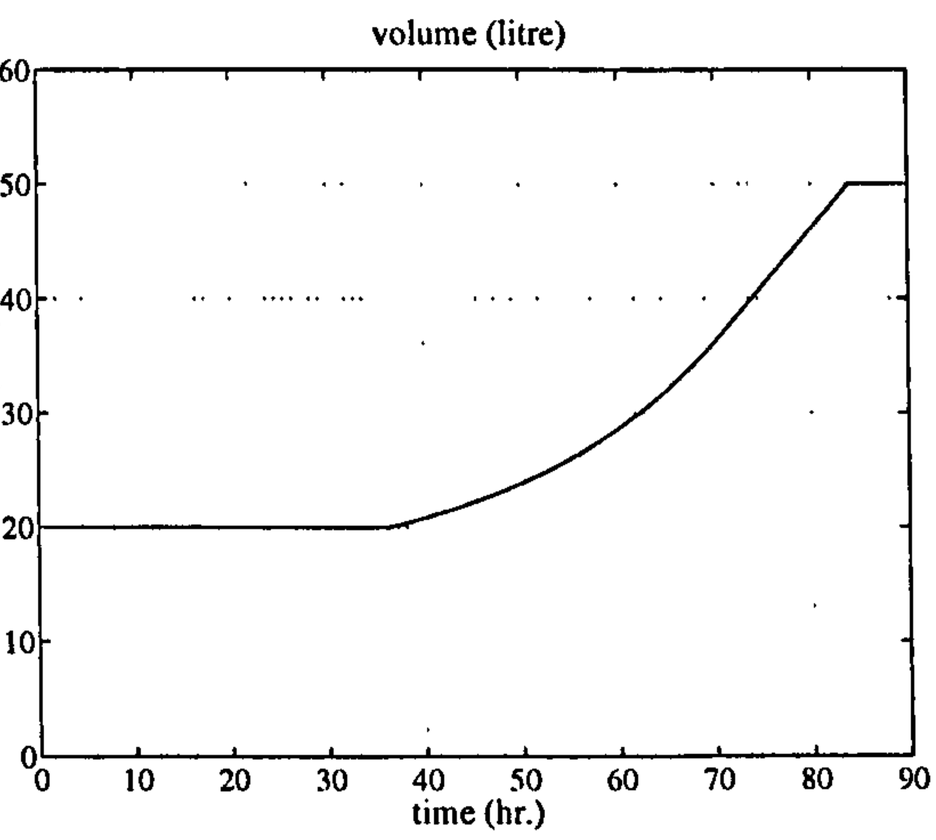

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Figure 5-5 Results of a primary metabolite production for CLOC (in perfect model)

5.2.1.2 Control simulation with model/process mismatch

We now examine the more realistic case where the model is not a perfect representation of the process. i.e. There is a process/model mismatch.

It is shown in the previous section that the CLOC method performs equally well as OLOFP in the case of a perfect model. However, consider a small error in parameter Y_{xs} to demonstrate where modelling has been inaccurate.

Parameter Y_{xs} in the process is still the same at 0.164. However, it is assumed that the parameter Y_{xs} that was obtained from modelling is different at 0.149 and 0.182 (10 % error). Since the important task is to keep the substrate concentration at 5 g/l , in this section, the initial substrate concentration in the process is at 5 g/l. Therefore the singular feed rate can start from the beginning for the OLOFP case. In this example, a nonlinear state feedback form for calculating the singular feed rate can be obtained as shown in Equation (5-6). This form of nonlinear state feedback is not generally obtained by this method. Therefore the comparison would be between those using purely open loop feed rate profile and nonlinear feedback in Equation. (5-6) for the OLOFP method and those obtaining from the CLOC method.

Considering first the nonlinear feedback in which feed rate is calculated from Equation (5-6) for the OLOFP method. It is shown in Figure 5-6 that the smaller value of parameter Y_{xs} (0.149) in the model results in the higher feed rate than the process needs and therefore increases the concentration of substrate in the reactor and makes the substrate concentration deviate from the singular substrate concentration (S_{sing} = 5 g/l). This deviation of substrate concentration results in the extension of operating time to 78 hr (compared with 72 hr for the CLOC case). This is due to the fact that the specific growth

rate is not maintained at the maximum ($\mu' = 0$). For the higher value of parameter Y_{xs} (0.182), the simulation is shown in Figure 5-7. The deviation of substrate concentration from the singular substrate concentration at 5 g/l can be explained by the fact that the error in the model results in the lower feed rate than the process really needs. The substrate concentration in the reactor then gradually reduces to zero. The zero level of substrate concentration also results in no growth in the fermenter (i.e. the specific growth rate becomes zero), which also results in no feed rate calculated from Equation (5-6). The operation has finished before the reactor is full due to no feed rate.

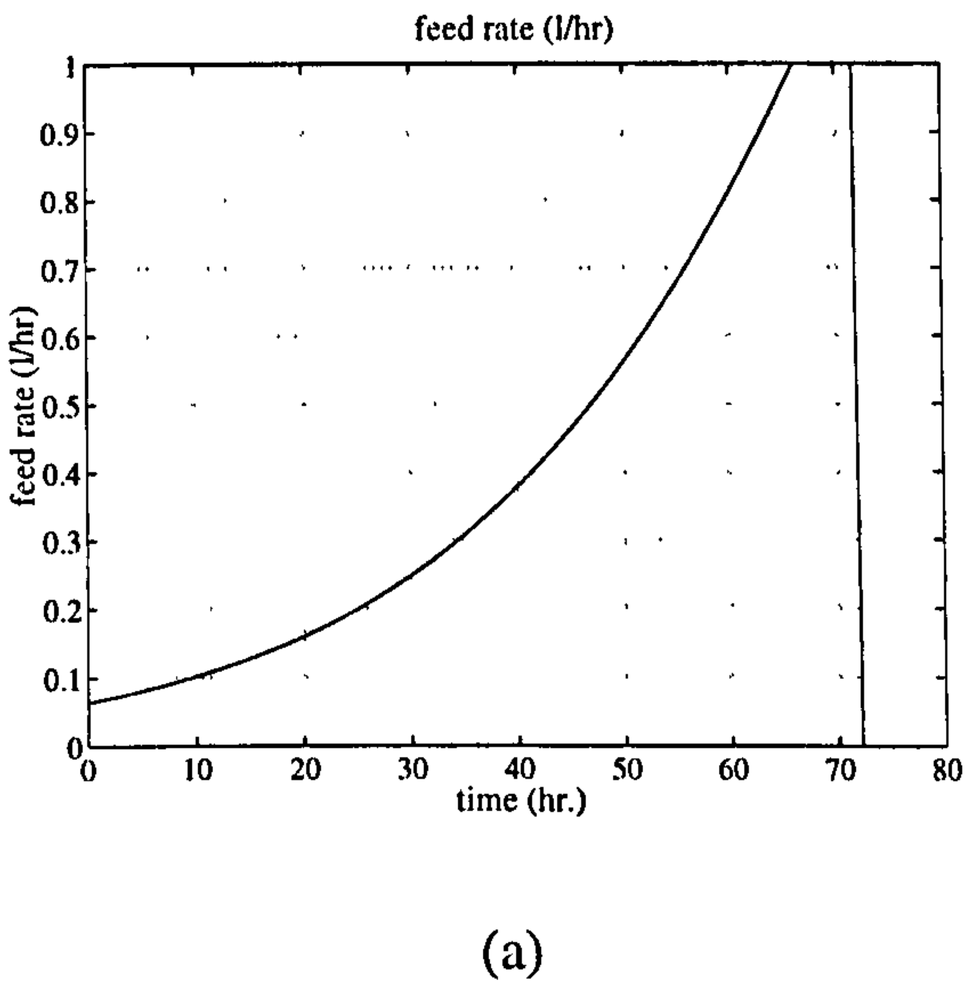
For the OLOFP method, where feed rate is purely pre-determined without any feedback, the simulation results are shown in Figure 5-8 and Figure 5-9 for the smaller and higher model parameter. The results also show the deviation of substrate concentration from the singular level. This can be explained by the fact that the error in parameter Y_{xs} causes the incorrect pre-determined optimal feed rate. The operating time lasts for 85 hours for the smaller parameter and for 77 hours for the higher parameter. However, since the feed rate is pre-determined, The substrate is fed until the reactor is full for both smaller and higher parameter cases.

Comparing with the CLOC method, it is shown in Figure 5-10 and Figure 5-11 that the error in Y_{xs} for both higher and smaller are well accommodated by the controller. The substrate concentration was kept at 5 g/l until the reactor is full. Although the final biomass concentration is similar to the ones obtained by using OLOFP method (with or without feedback), the time to reach a maximum biomass concentration is shorter (72 hr). The same amount of biomass obtained can be explained by the fact that we fed the fermenter with an equal amount of substrate and when it is all converted to biomass, it gives the same amount of biomass.

In this example, the deterioration of performance in the OLOFP method (both purely open loop and feedback) comparing with the CLOC method demonstrates the advantages of the proposed control scheme. The operating time for the CLOC method is at 72 hr, which is the shortest operating time comparing to the OLOFP method. If there are other inaccurately modelled parameters or any disturbance particularly in the concentration of substrate in the feed stream, the deterioration of performance in OLOFP would be more significant. The effect of error in other model parameters will be discussed again later. This advantage can also be counted as the result of better understanding of the process since we have already known that substrate concentration is one of factors that govern the bioreaction rate in the fermentation process.

In summary, the CLOC method `provides better performance than the OLOFP method for the primary metabolite process under the plant/model mismatch case. This is demonstrated by the shortest operating time of the CLOC method (72 hr in Figure 5-10 and 5-11) comparing with the OLOFP method (77 hr in Figure 5-9, 85 hr in Figure 5-8 and 78 hr in Figure 5-6).

In the next section, we will examine and compare the performance of the CLOC and OLOFP methods for a secondary metabolite production process.



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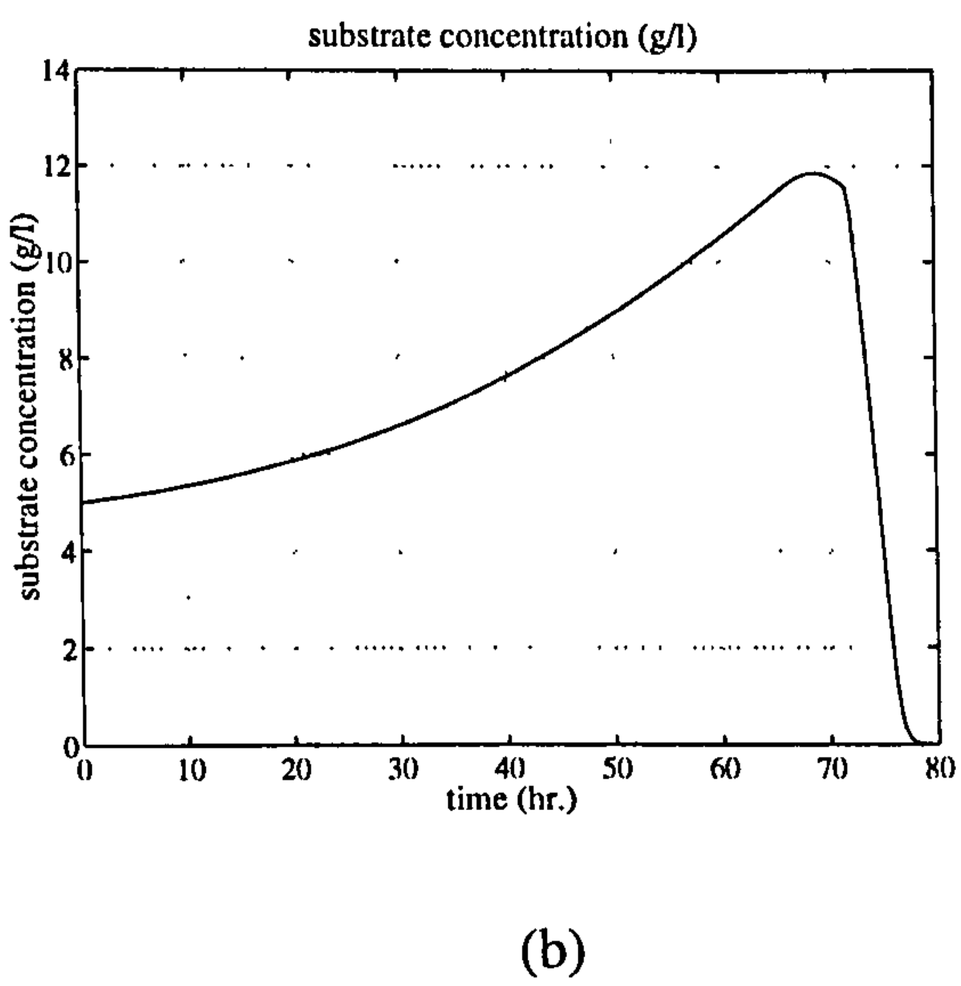

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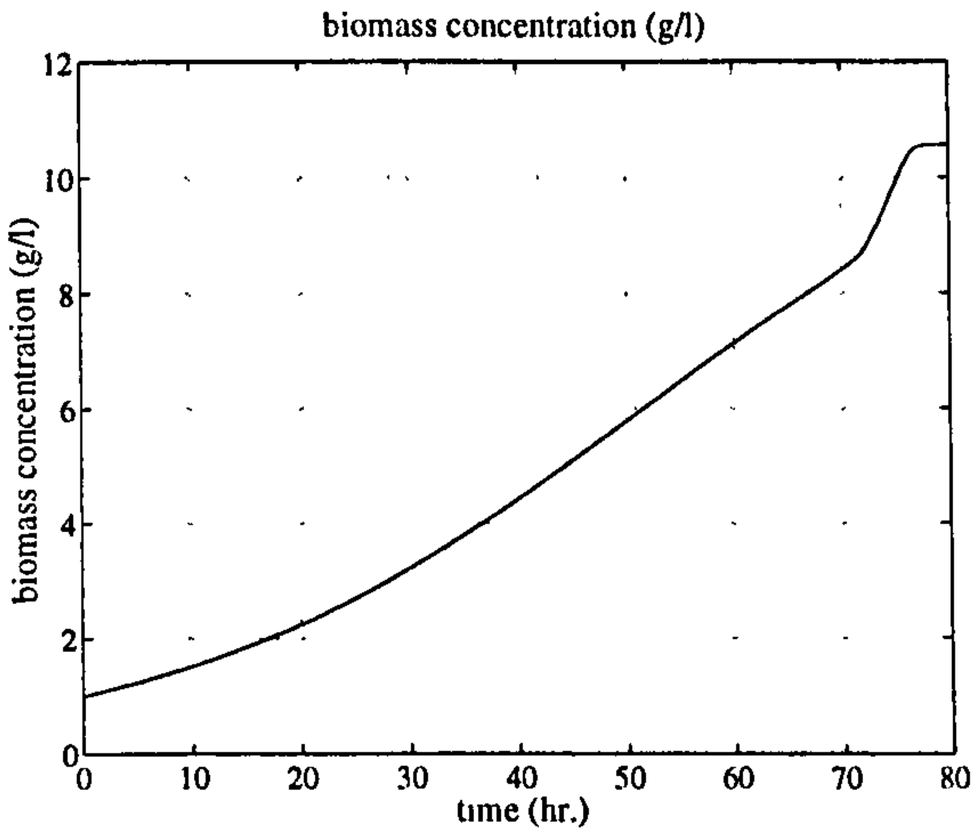

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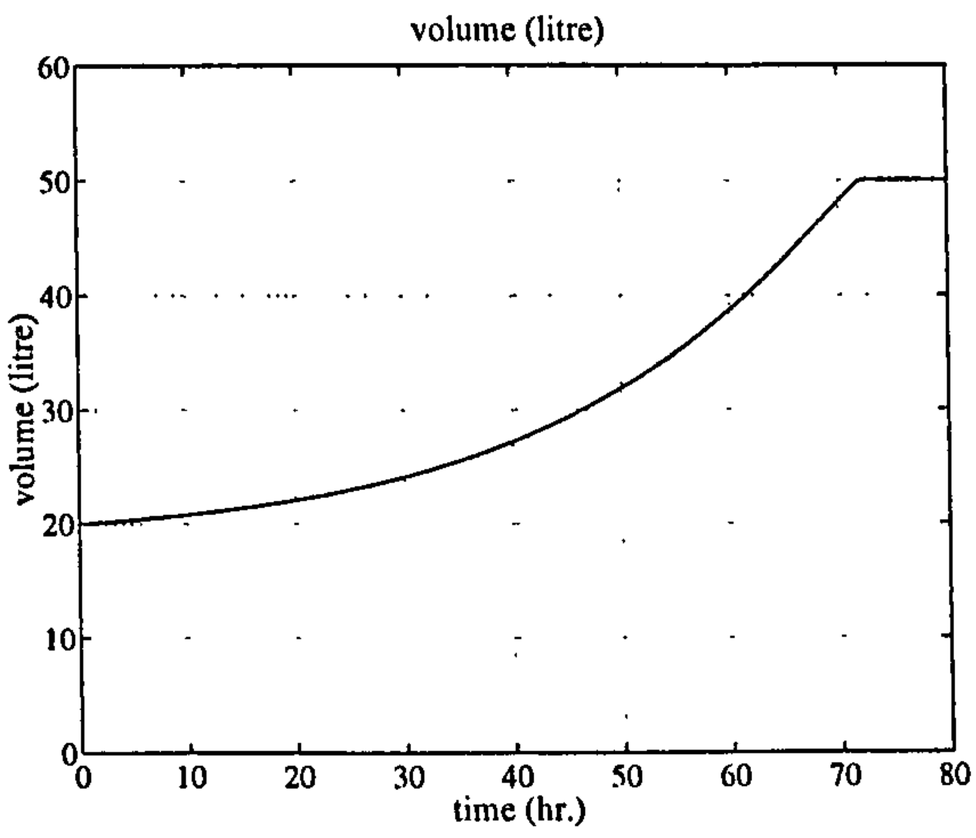

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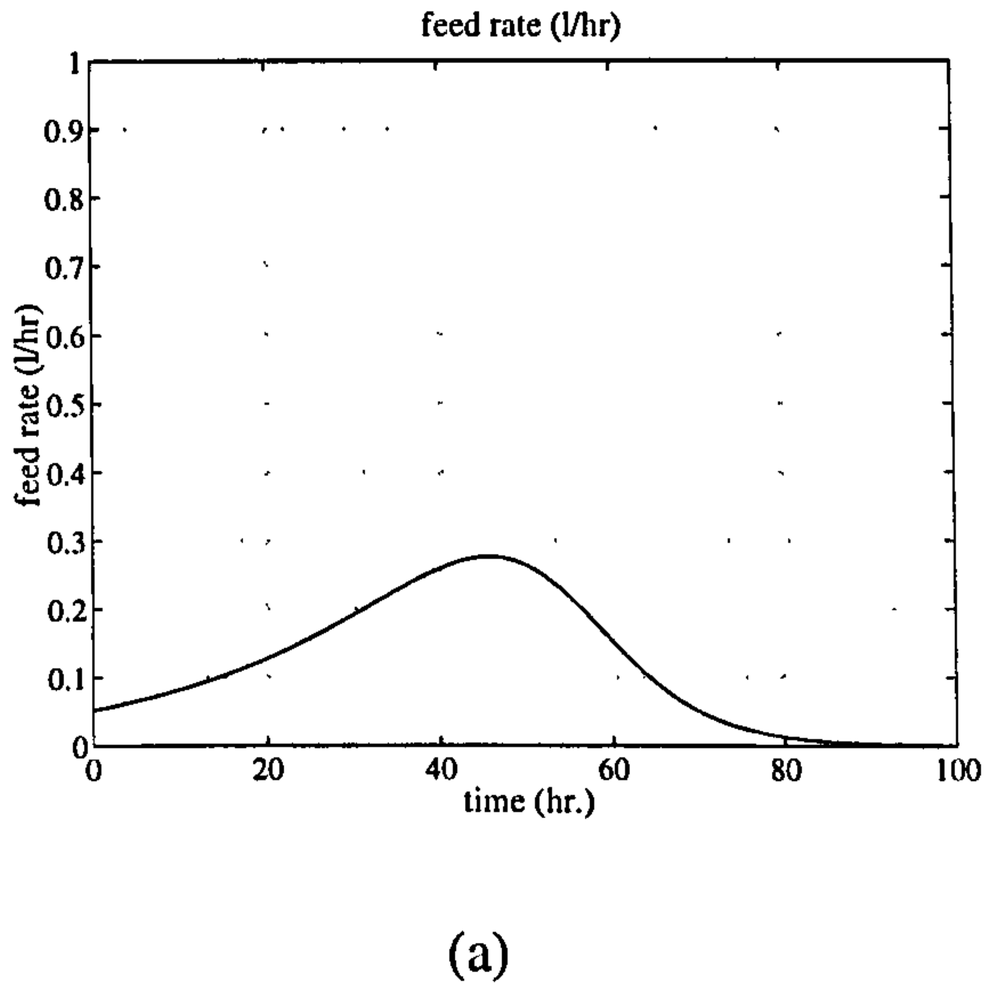
Figure 5-6 Simulation results for the OLOFP method using Equation (5-6) for calculating feed rate (model parameter is smaller than the plant parameter). (primary metabolite production)



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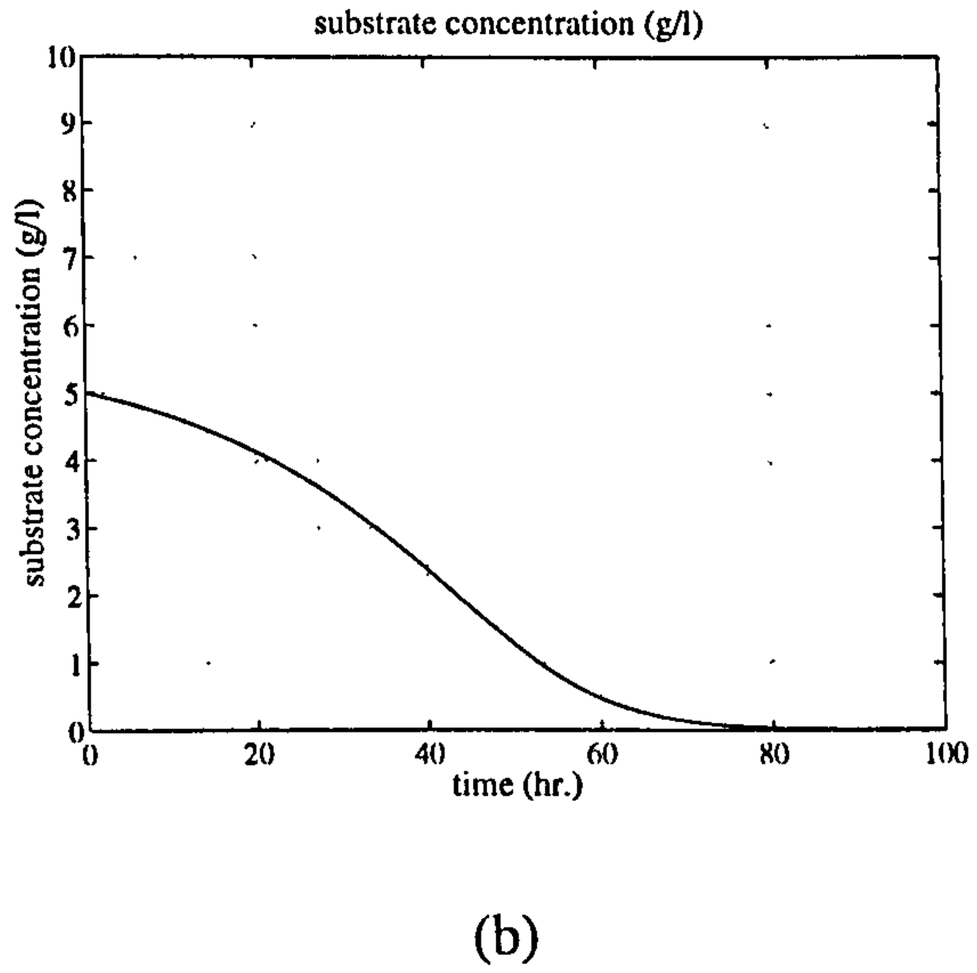

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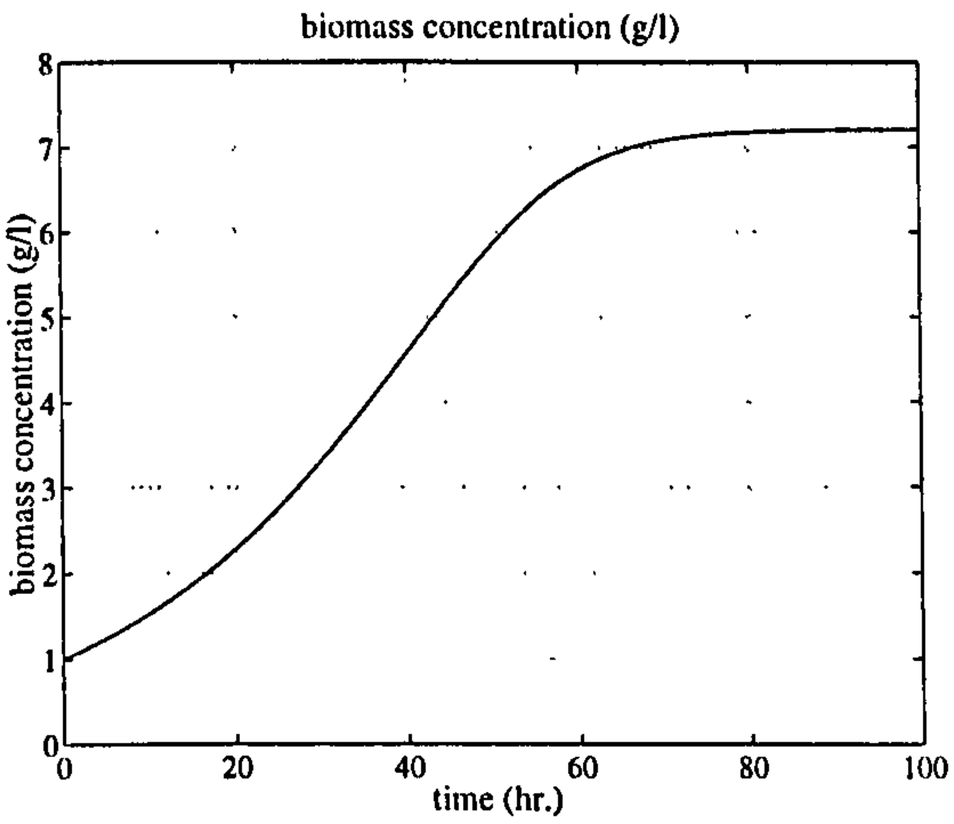

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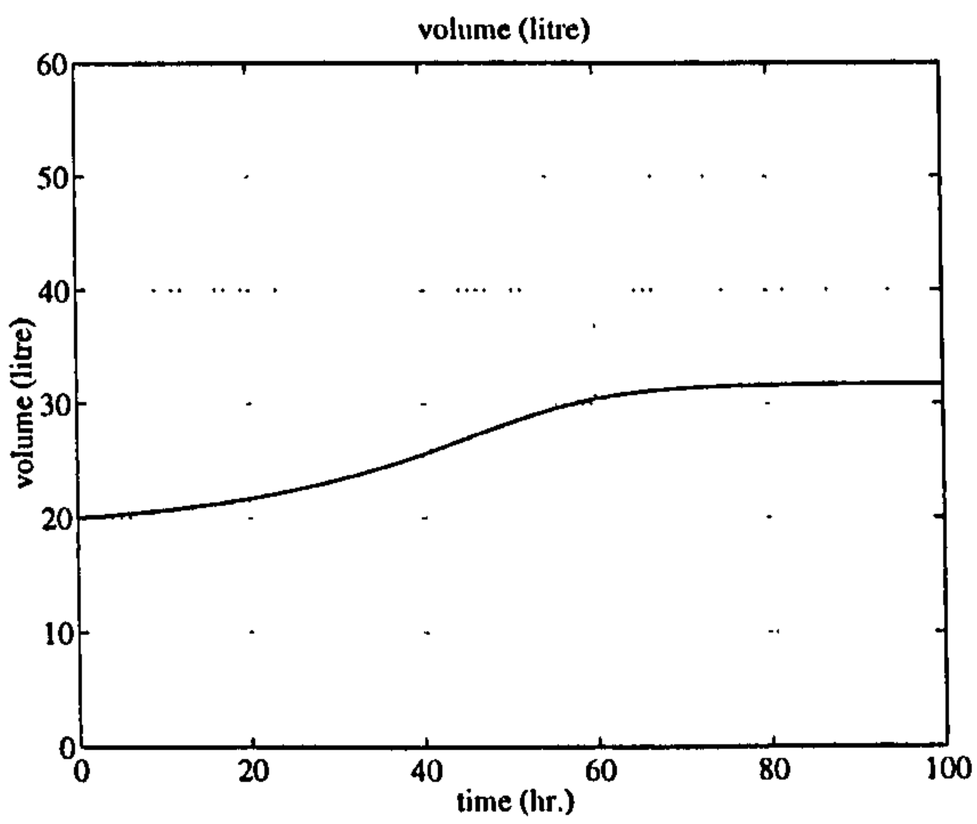

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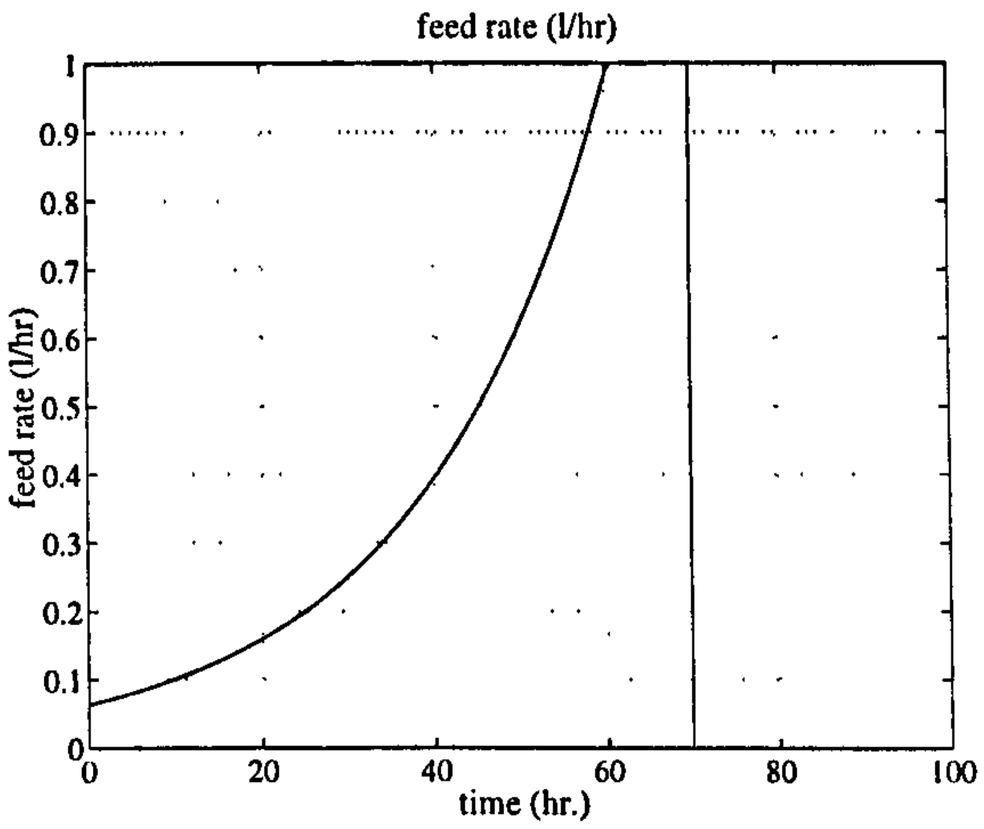
Figure 5-7 Simulation results for the OLOFP method using Equation (5-6) for calculating feed rate (model parameter is higher than the plant parameter). (primary metabolite production)



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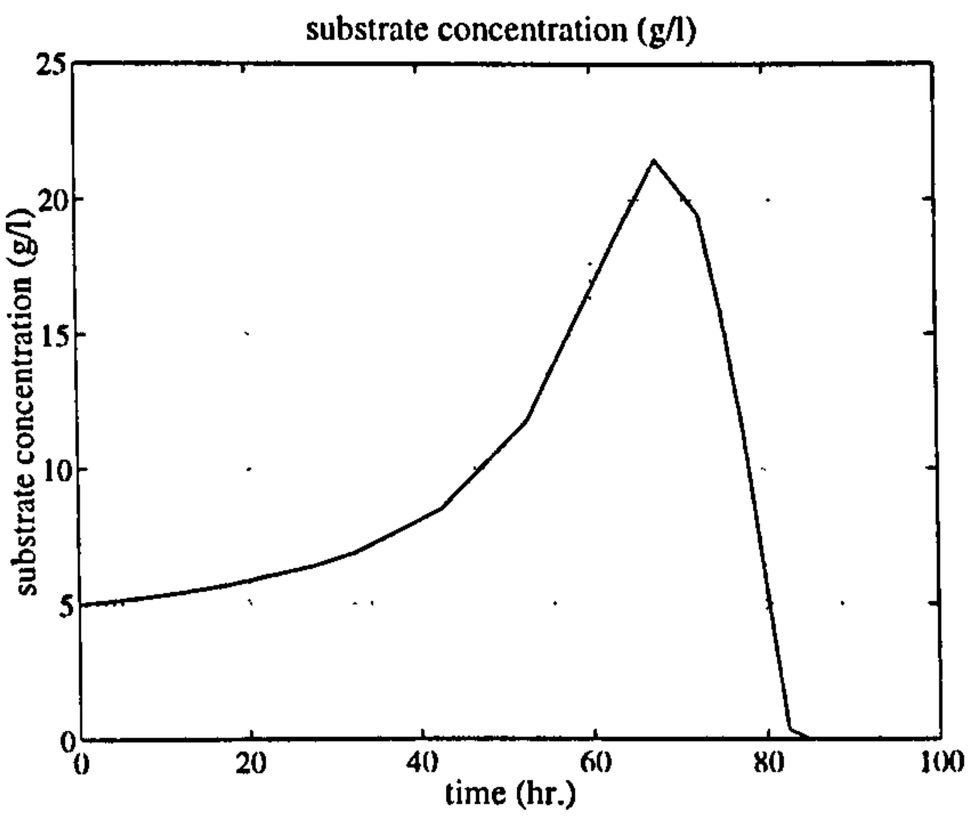

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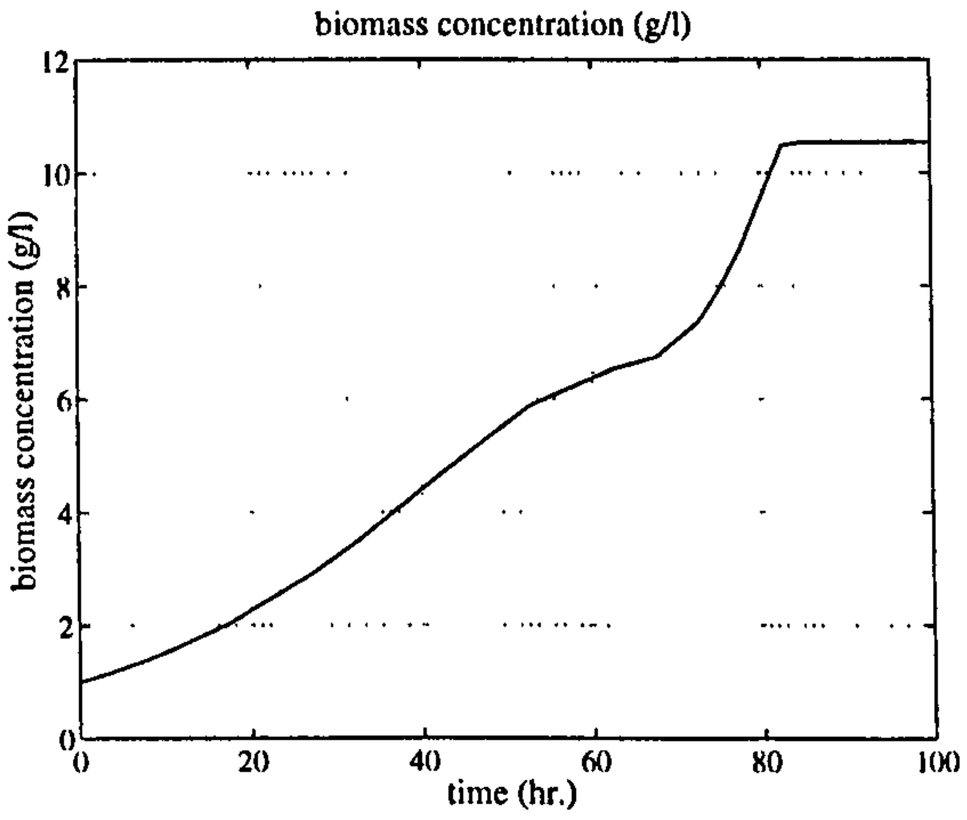

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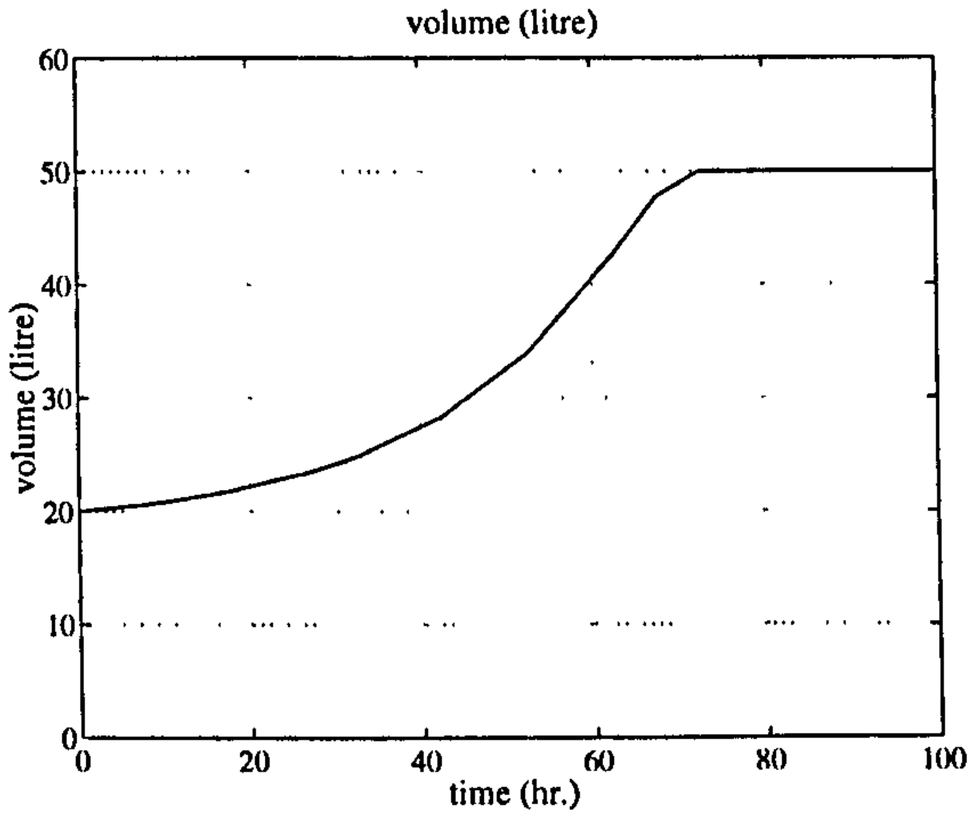
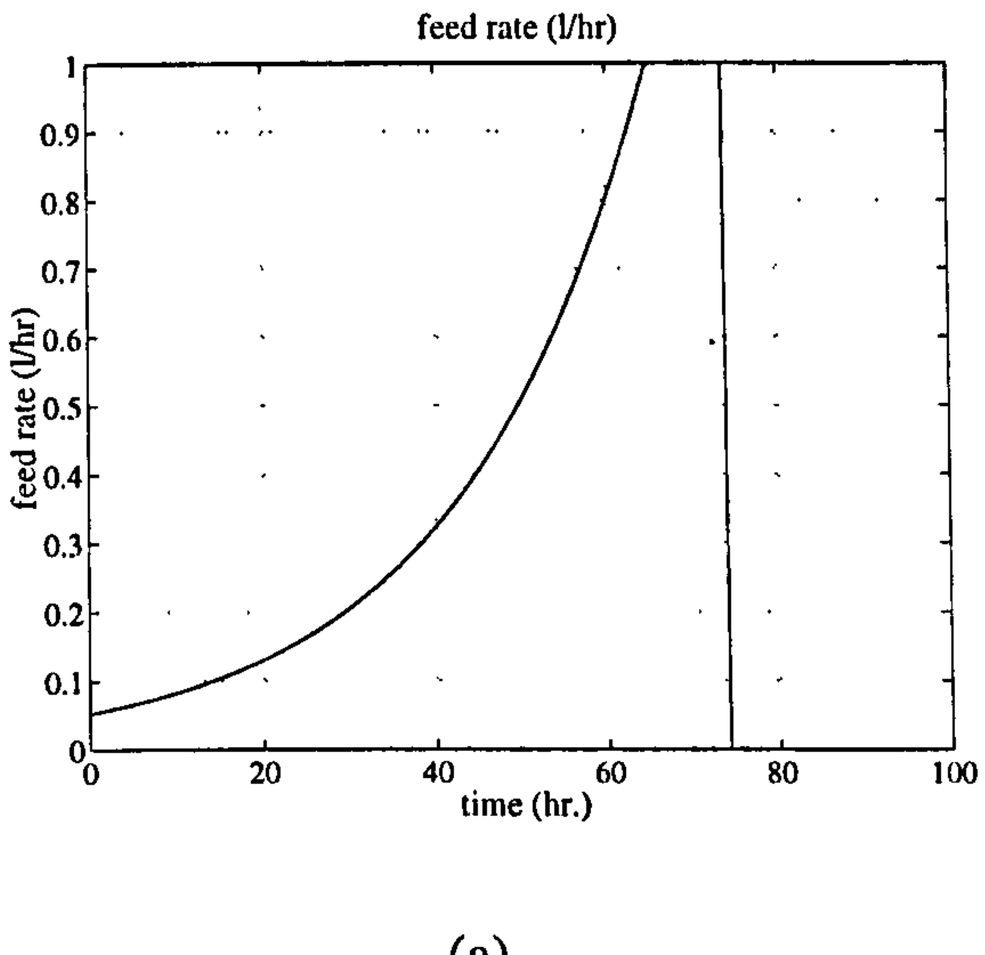


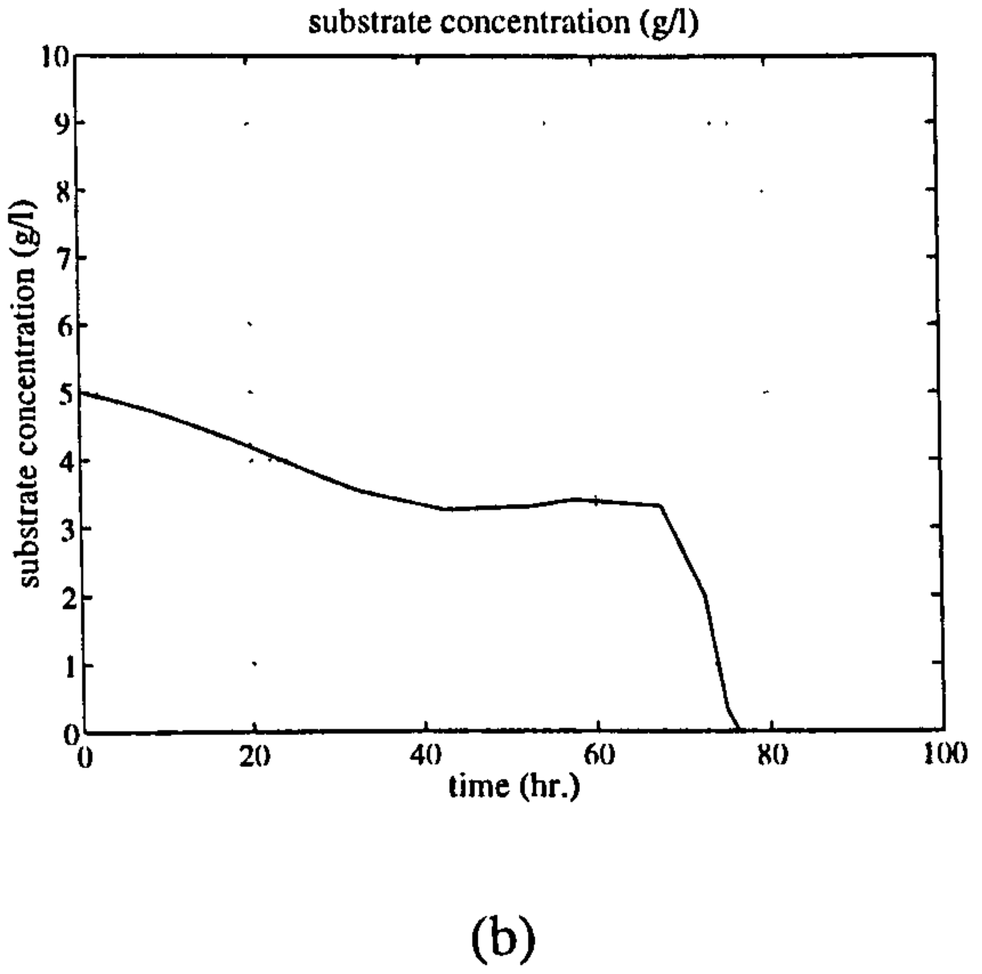
(d)

Figure 5-8 Simulation results for the OLOFP method using pre-determined feed rate (model parameter is smaller than the plant parameter).

(primary metabolite production)



(a)



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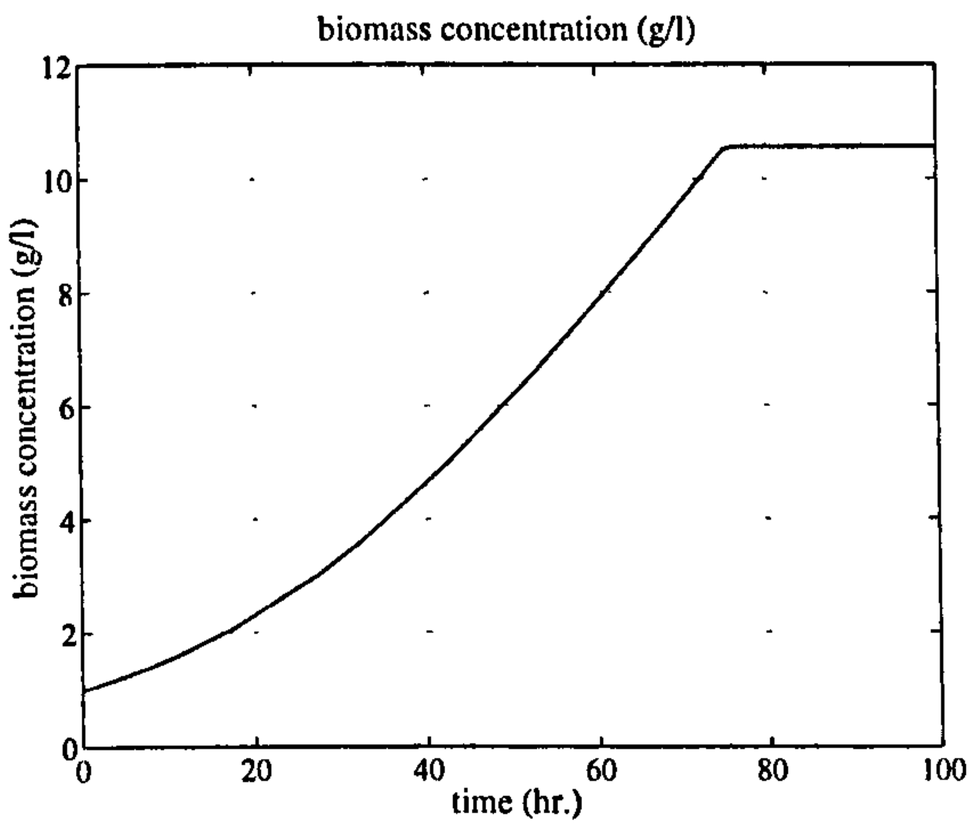

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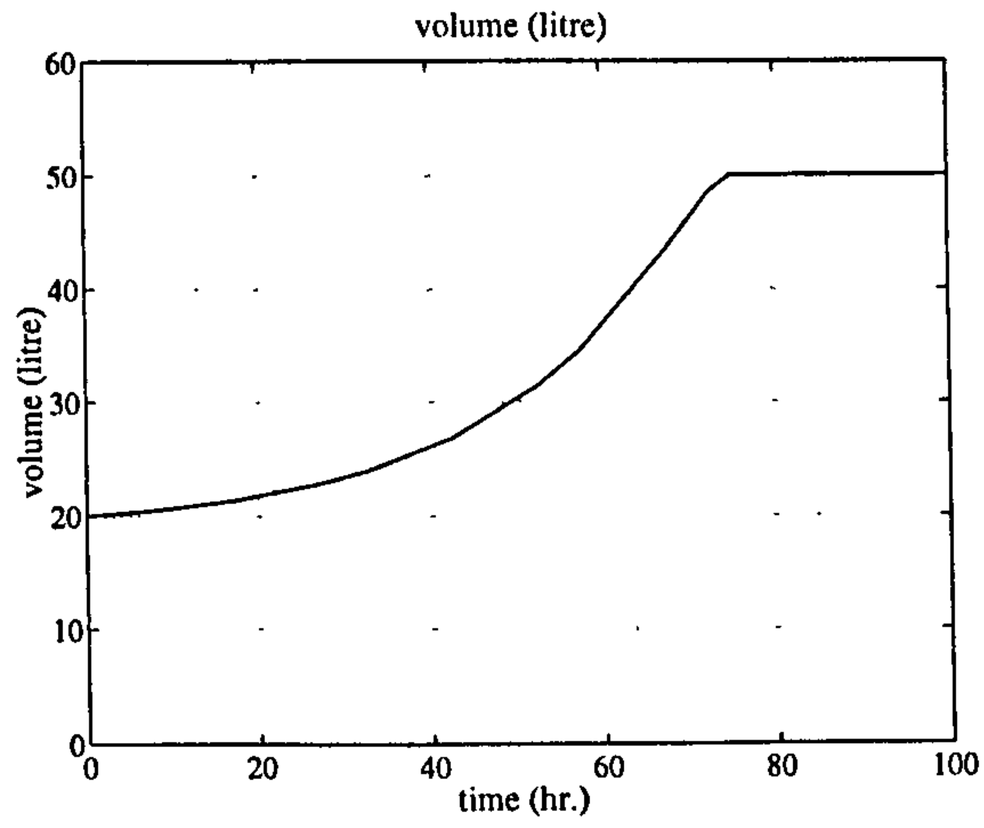
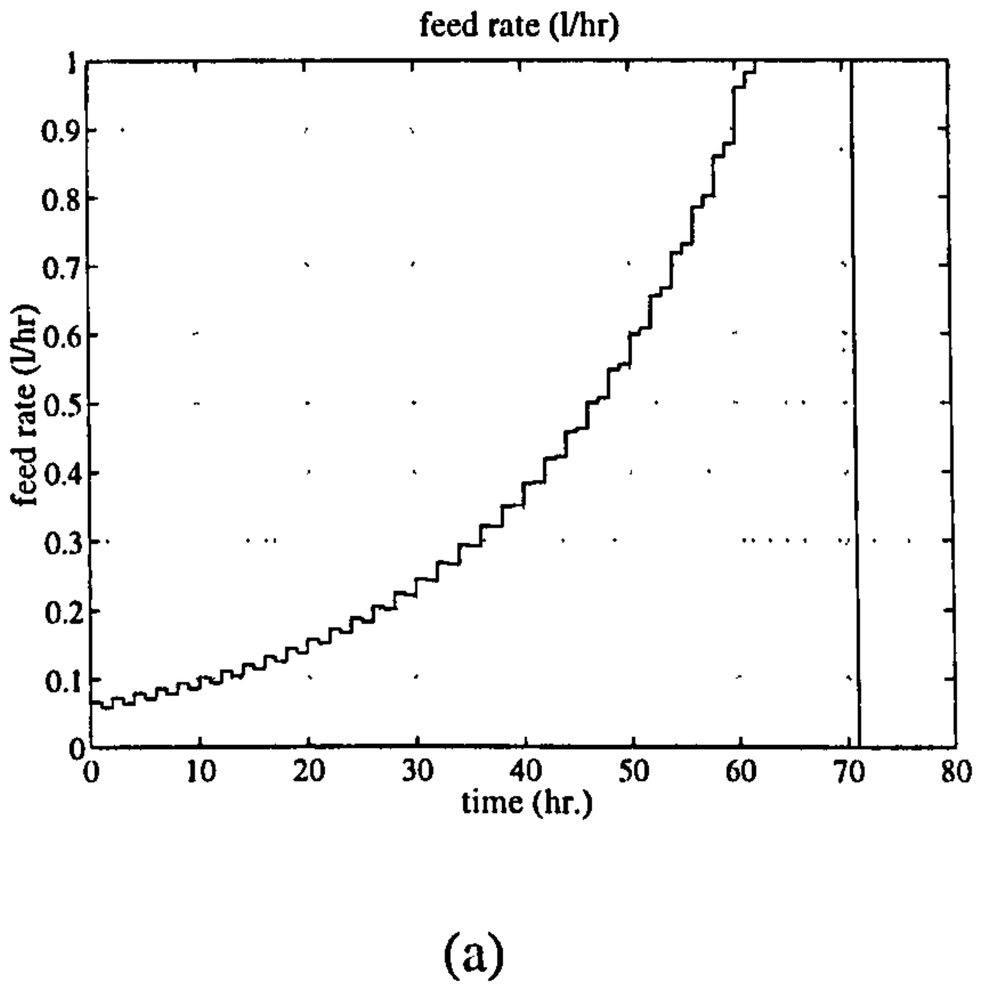


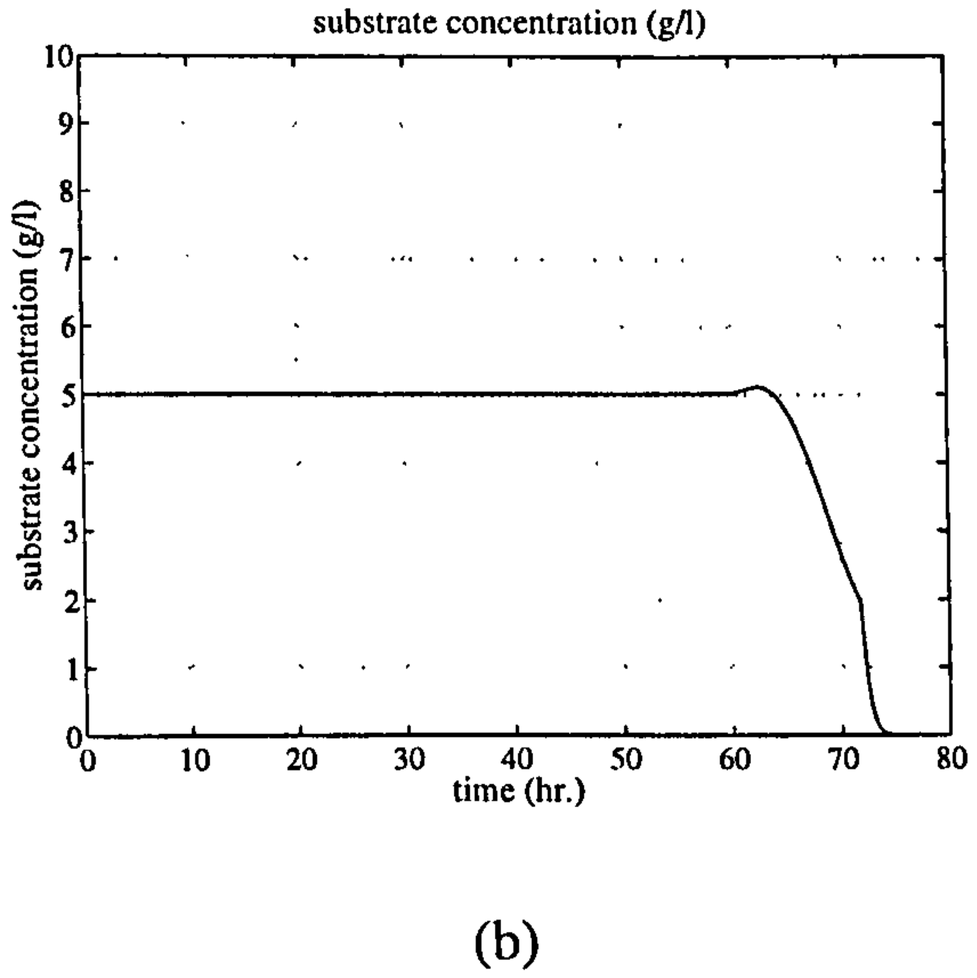
(d)

Figure 5-9 Simulation results for the OLOFP method using pre-determined feed rate (model parameter is higher than the plant parameter).

(primary metabolite production)



(a)



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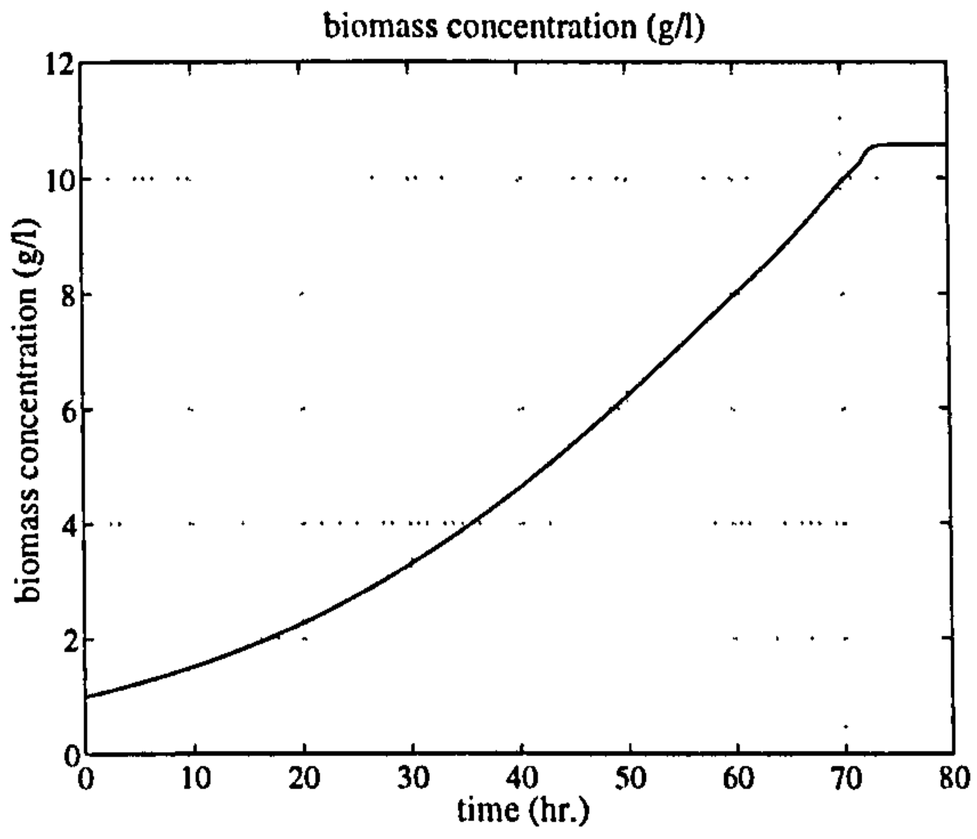

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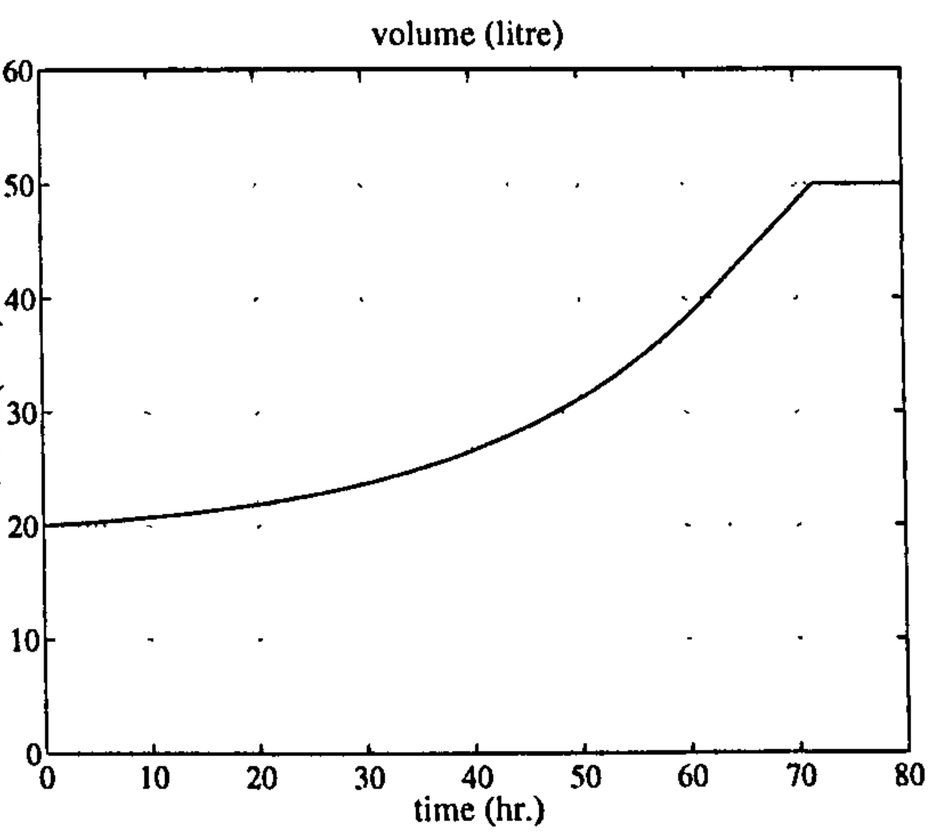


(d)

Figure 5-10 Simulation results for the CLOC method (model parameter is smaller than the plant parameter).

(primary metabolite production)

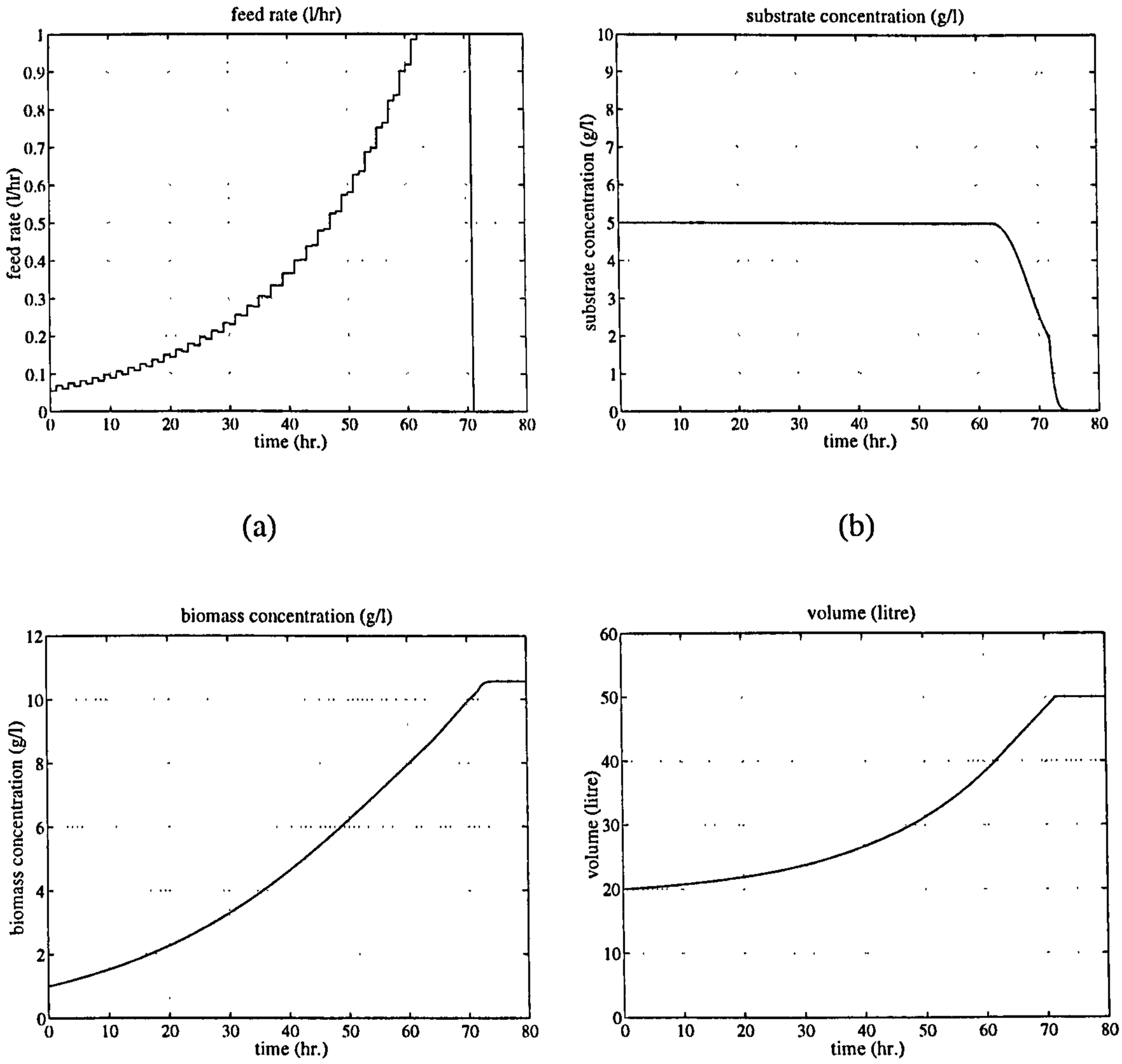


Figure 5-11 Simulation results for the CLOC method (model parameter is higher than the plant parameter).

(primary metabolite production)

5.2.2 Secondary metabolite production

The fed-batch fermentation models for a secondary metabolite production are those in Chapter 2 (refer to equation (2-28) to (2-32)) and are written here as:

$$\frac{dX}{dt} = \mu X - DX \tag{5-7}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{xs}}\mu X + D(S_f - S) \tag{5-8}$$

$$\frac{dP}{dt} = \pi X - DP \tag{5-9}$$

$$\frac{dV}{dt} = F \tag{5-10}$$

$$D = \frac{F}{V} \tag{5-11}$$

where the kinetic reaction of the specific growth rate (μ) and specific product formation rate (π) are in the substrate inhibition form as shown in the following:

$$\mu = \frac{\mu_{max} S}{K_s + S + S^2/K_i} \tag{5-12}$$

$$\pi = \frac{\pi_{max} S}{K_{\pi s} + S + S^2/K_{\pi i}} \tag{5-13}$$

The relationship between the specific product formation rate (π) and substrate concentration (S) is shown in Figure 5-12 and the parameter values used for the simulation are in Table 5-3.

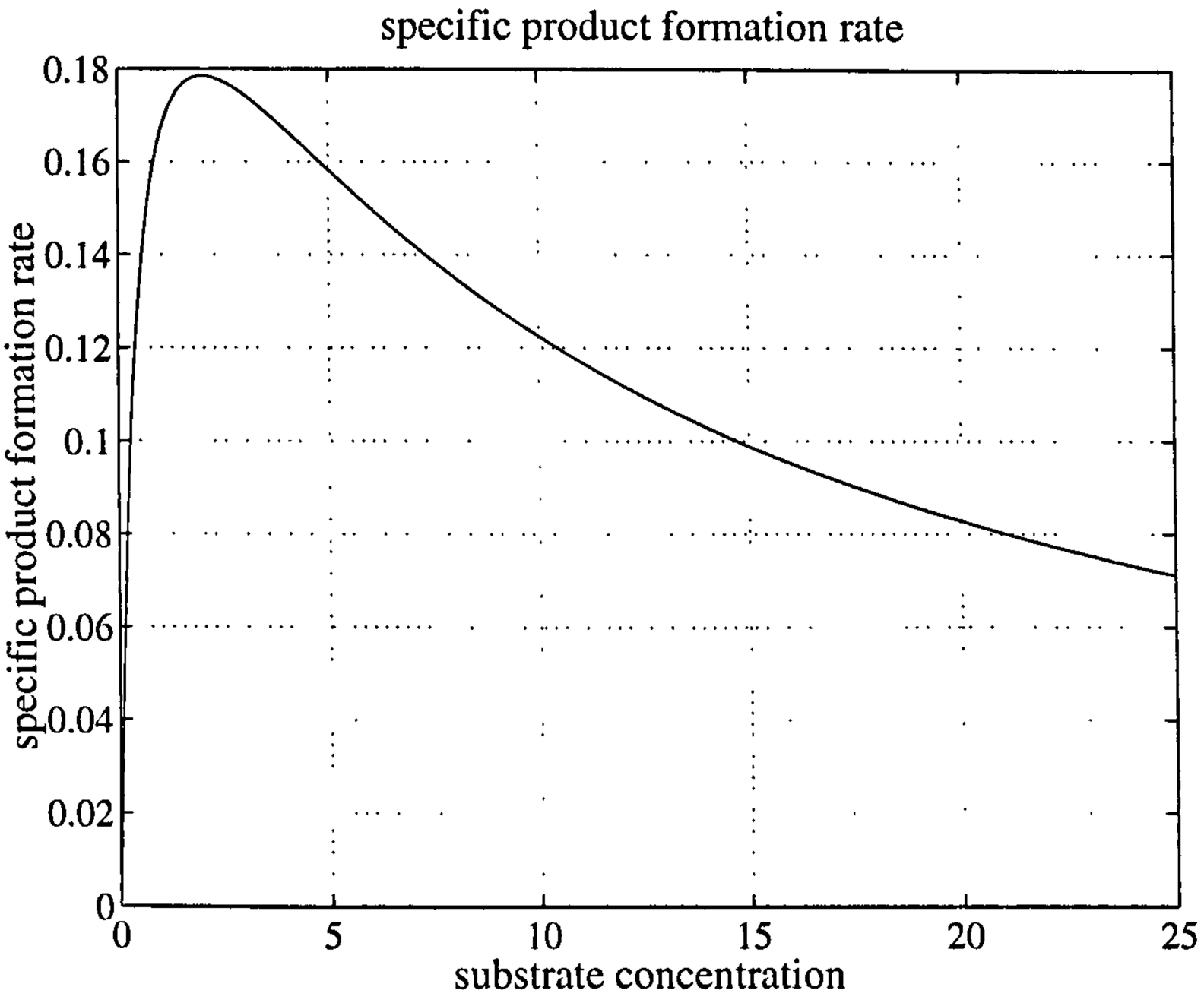


Figure 5-12 Relationship between the specific product formation (π) and substrate concentration (S)

Table 5-3 Parameters used in the simulation for a secondary metabolite production

Parameter	Value	Unit
μ_{max}	0.10	(g biomass / (g biomass * hr))
K_s	3.0	(g substrate / litre)
K_i	8.34	(g substrate / litre)
Y_{xs}	0.164	(g biomass / g substrate)
π_{max}	0.25	(mg product / (g biomass * hr))
$K_{\pi s}$	0.4	(g substrate / litre)
$K_{\pi i}$	10	(g substrate / litre)
X(0)	1.0	(g biomass / litre)
S(0)	4.6	(g substrate / litre)
V(0)	20	(litre)
V(tf)	50	(litre)
S_f	100	(g substrate /litre)

where

μ_{max} is the maximum specific growth rate

π_{max} is the maximum specific product formation rate

K_s, K_i, $K_{\pi s}$ and $K_{\pi i}$ are rate constants

Y_{xs} is yield of biomass from substrate

X(0), S(0) and V(0) are initial condition of biomass and substrate concentration, and culture volume

$V(t_f)$ is final culture volume

S_f is substrate concentration in the substrate feed stream

For the secondary metabolite production considered in the previous chapters, there were two cases to be considered - include or not include the cost of operating time in the objective function (J).

$$J = P(t_f)$$

$$J = P(t_f) - \varepsilon \int_{t_0}^{t_f} dt$$

The cost of operating time is used to weight the profit from final product concentration with the expense of time during process operation and presented in term of cost factor (ε) in the objective function. The higher cost factor, the more costly of operating time and the higher requirement to shorten the process.

The determination of optimal feed rate and optimal substrate concentration profiles for both cases of objective functions were presented in Chapter 3 (Section 3.2.2) and Chapter 4 (Section 4.2.2). Considering first the objective function without the cost factor (ε). For the OLOFP method, the singular feed rate would maintain the substrate concentration at the constant level (refer to Equation (3-62)) during the singular period. The condition for determining this singular substrate concentration was obtained from Equation (3-63). For

the CLOC method, the optimal substrate concentration was kept constant at the level, which maximises the ratio between the specific product formation rate (π) and the specific growth rate (μ) (refer to Equation (4-31)). Note that Equation (3-63) and (4-31) are similar.

Since the substrate concentration is kept constant, the results from the previous section for a primary metabolite process can also be applied here and we will therefore omit this case and consider only the second one in which the objective function includes the cost factor. This case is more important because most of the processes are usually operated under some production time constraint.

We would start from the beginning at the singular period in the OLOFP case and at the optimal substrate concentration profile in the CLOC case since these parts are at the optimal as mentioned earlier. Also, there is no effect of feed rate constraints on this period. The substrate profile in both conditions are similar (refer to Equation (3-58) and Equation (4-27)) and is written here as Equation (5-14),

$$\dot{S} = -\frac{\mu'(\pi'\mu - \pi\mu')}{(\mu'\pi'' - \pi'\mu'')} \tag{5-14}$$

Note that the substrate profile in (5-14) is only a function of substrate concentration and shown in Figure 5-13. As biomass also has effect on production, the biomass concentration that corresponds to the optimal substrate profile was also derived in Chapter 3 and 4 (refer to Equation (3-57) and Equation (4-26)) and is written here as:

$$X = -\frac{\mu'\varepsilon}{(\pi'\mu - \pi\mu')} \tag{5-15}$$

The relationship between the biomass and substrate concentration during the optimal period at different cost factors is shown in Figure 5-14. The far left line shows the smaller cost factor, while the far right shows the bigger cost factor.

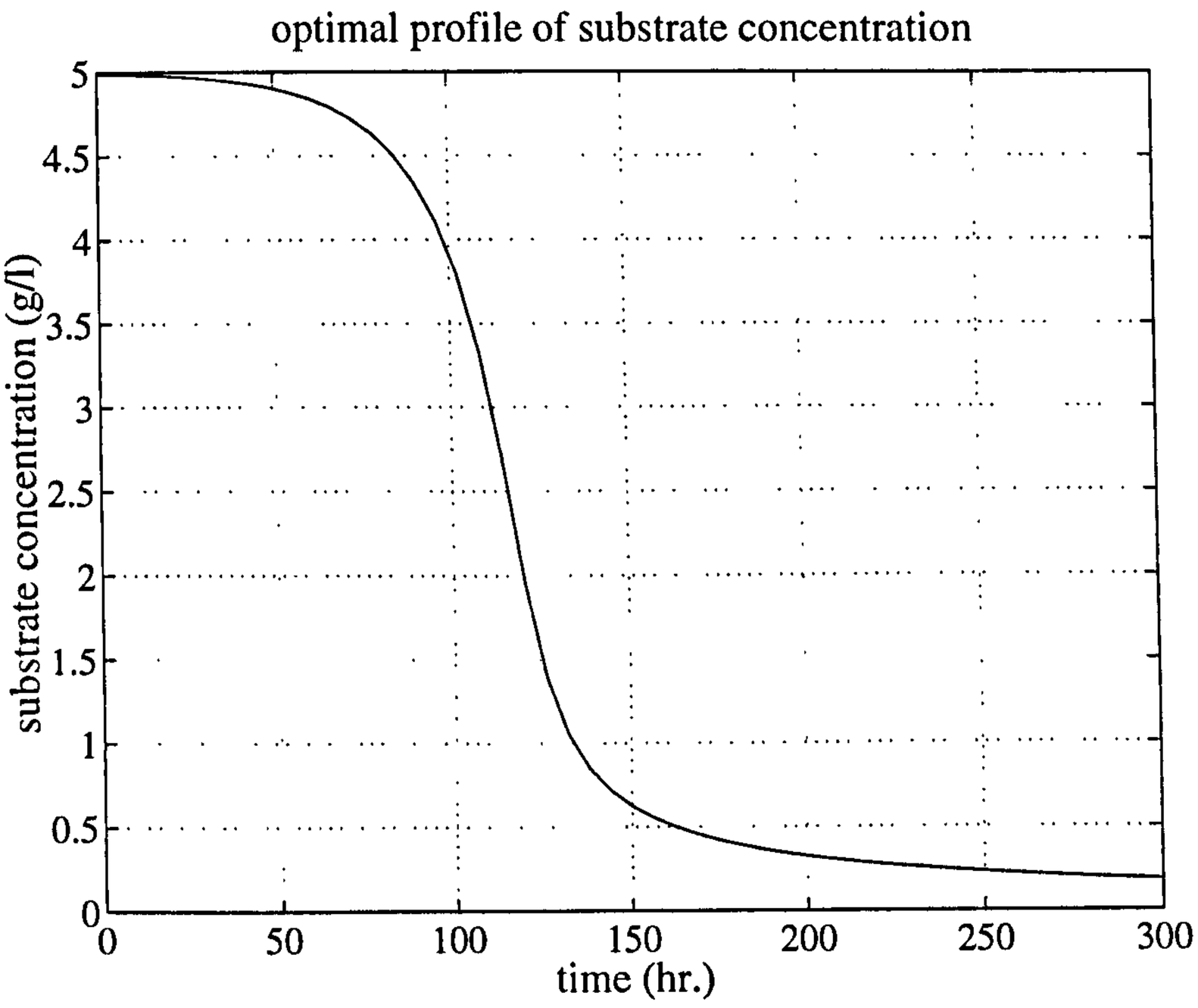


Figure 5-13 Relationship between optimal substrate concentration and time

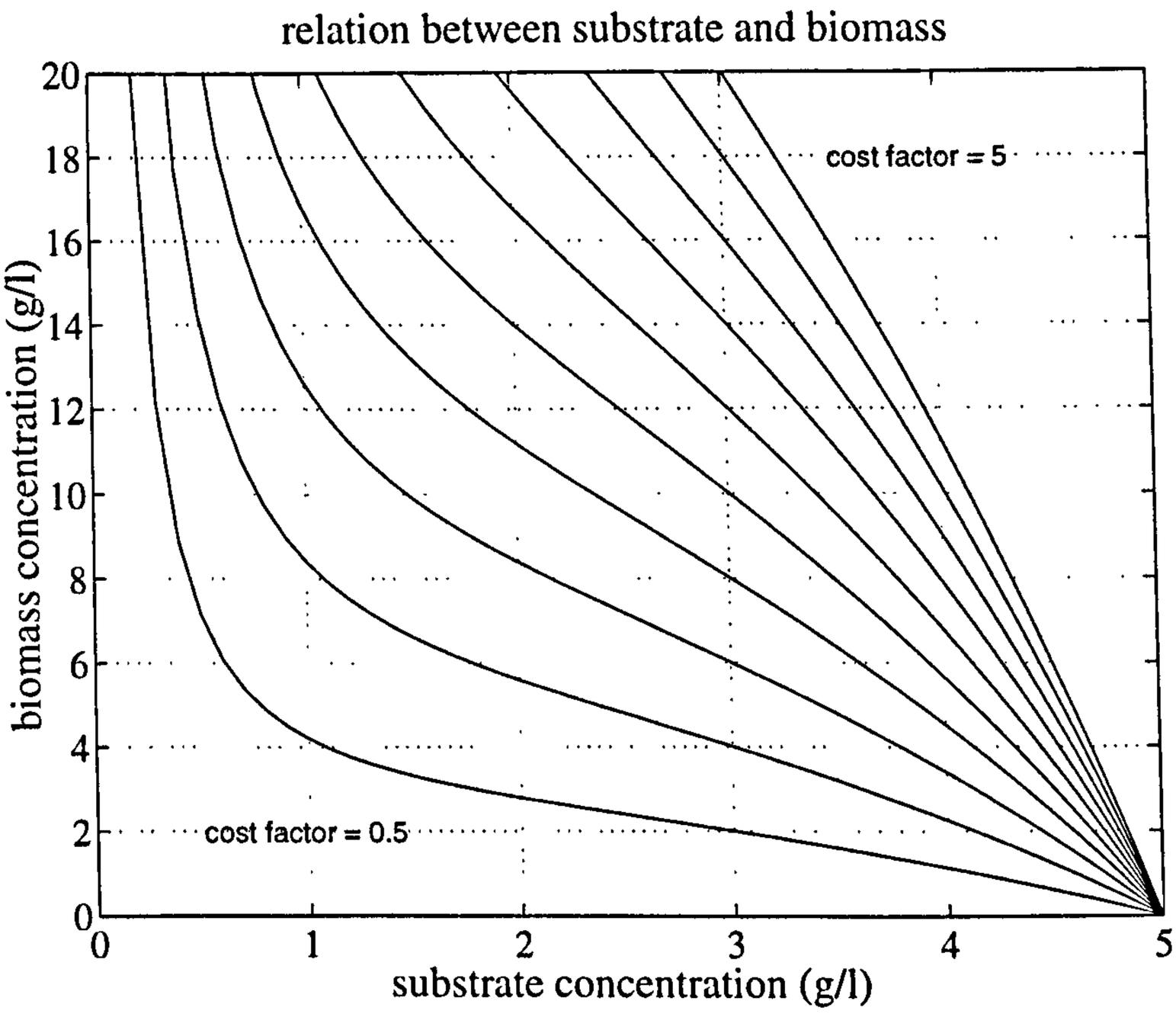


Figure 5-14 Relationship between biomass and substrate concentration at different cost factors (ε)

The lines, from left to right, illustrate the effect of the cost factor (ε) that increases from 0.5 to 5 on the relationship between substrate and biomass concentration during the optimal period. It is followed from Section 3.2.3 and 4.2.3 in the previous chapters that as the cost factor increases, the process is shorter by faster growing of biomass and reduce the final product. This will be discussed more with some simulations in the next section.

For The OLOFP method, the optimal feed rate can be obtained from Equation 3-48,

$$\frac{\partial H}{\partial F} = -\frac{\lambda_X X}{V} + \lambda_V + \frac{\lambda_S (S_f - S)}{V} - \frac{\lambda_P P}{V} = \Psi \qquad (3\text{-}48)$$

with the following conditions:

if $\Psi < 0$ then $F = 0$

if $\Psi > 0$ then $F = F_{max}$

if $\Psi = 0$ then $F = F_{sing}$

The singular feed rate (F_{sing}) can be obtained from Equation (3-59) and is written here as:

$$F_{sing} = \frac{V}{(S_f - S)}\left(\frac{\mu X}{Y_{xs}} + \frac{\mu'(\pi'\mu - \pi\mu')}{(\pi'\mu'' - \pi''\mu)}\right) \qquad (5\text{-}16)$$

For the CLOC case, the optimal substrate concentration profile can be obtained from Equation (4-27):

$$\dot{S} = -\frac{\mu'(\pi'\mu - \pi\mu')}{(\mu'\pi'' - \pi'\mu'')} \qquad (4\text{-}27)$$

In simulation of the CLOC method, the following parameters are used in the nonlinear model predictive controller:

sampling time - 1 hr.

prediction horizon - 5 hr.

control horizon - 3 hr.

The cost factor (ε) has an effect on the objective function and therefore process trajectories. The cost factor that equals one is used here for the following comparison simulations.

5.2.2.1 Control simulation with perfect model

The simulation results for the OLOFP and CLOC methods are shown in Figure 5-15 and Figure 5-16 respectively. (In each Figure 5-15 to Figure 5-22, (a) indicates substrate concentration, (b) substrate feed rate, (c) product concentration, (d) biomass concentration and (e) culture volume.) The comparison between operating time and secondary metabolite product obtained is shown in Table 5-4. It can be seen that for the perfect model case, both methods give similar results.

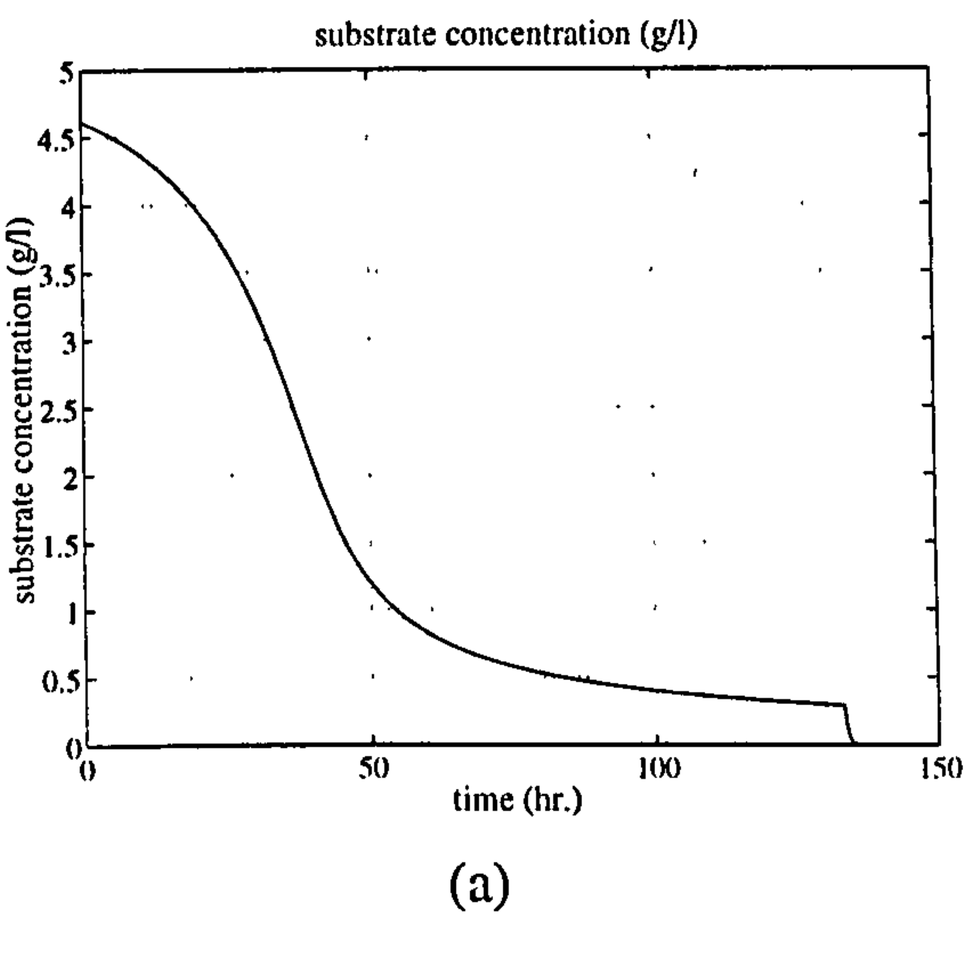
Table 5-4 Comparison between OLOFP and CLOC in perfect model for a secondary metabolite process

Control Method	OLOFP	CLOC
Finish time (hr.)	134	134
Maximum product (mg/l)	84.43	84.42
Maximum biomass (mg/l)	10.54	10.54

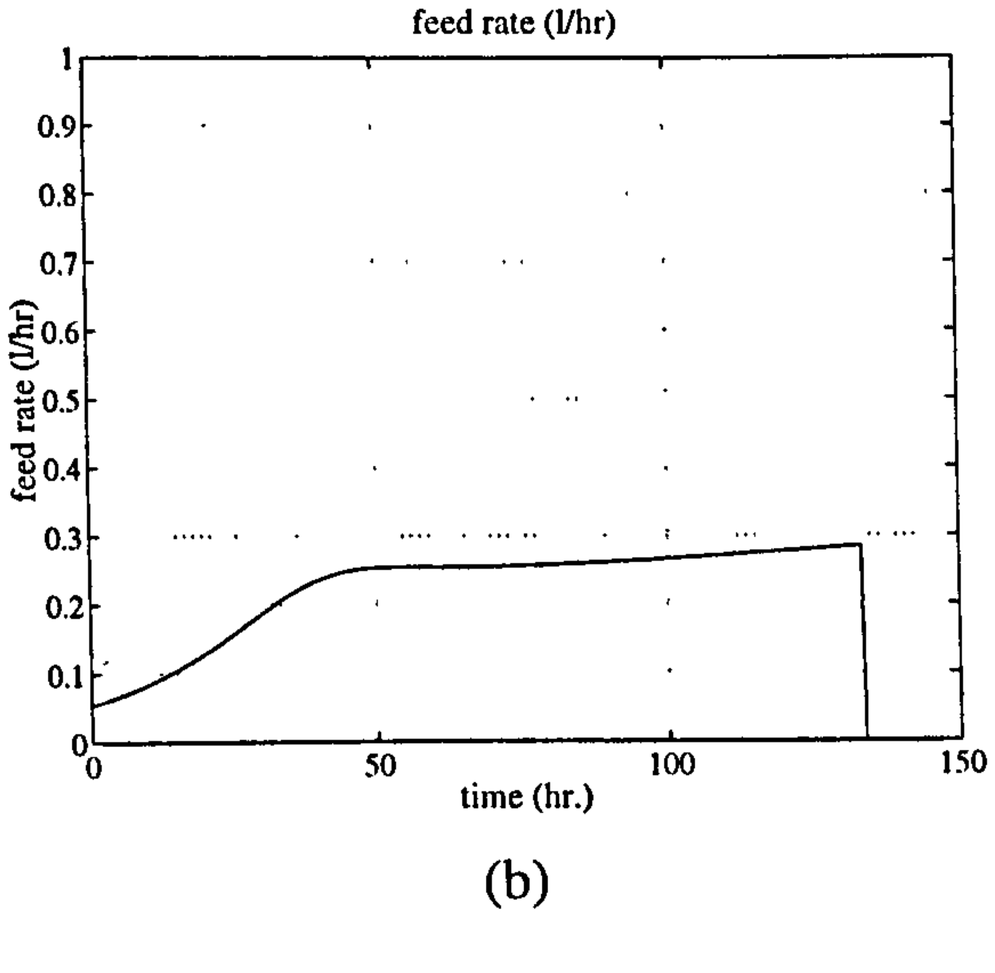
From the simulations, the process starts with the singular period. The singular feed rate results in the profile of substrate concentration as shown in Figure 5-15 (a) which is similar to the optimal substrate concentration calculated from Equation (4-27) and shown

in Figure 5-13. This optimal substrate profile determined from Equation (4-27) is used as a reference profile for a nonlinear model predictive control to follow. The optimal substrate profile is shown as a dashed line in Figure 5-16 (a). It can be seen that the controller tracks the substrate profile very well. The substrate concentration deviates from the optimal profile at around 134 hours because the culture volume reaches the maximum, which results in no feed rate and decreasing in substrate concentration as shown in the figure. For the OLOFP method, there is no substrate concentration profile to follow. The singular feed rate is determined by Equation (5-16) until the reactor is full and the singular period is then ended. Without any feed rate, the substrate concentration begins decreasing and eventually depleted. For this cost factor ($\varepsilon = 1$), most of the whole operation is operated under the singular period for the OLOFP method and feed rate does not reach the maximum constraint. For the CLOC method, feed rate is sufficient to provide the substrate concentration following the desired trajectory without any saturation occuring.

For the prefect model case, the same phenomena can be obtained from both methods however with a different interpretation. The simulations show that both methods provide a similar performance for the secondary metabolite production process. In next subsection, a case for plant/model mismatch is considered.



(a)



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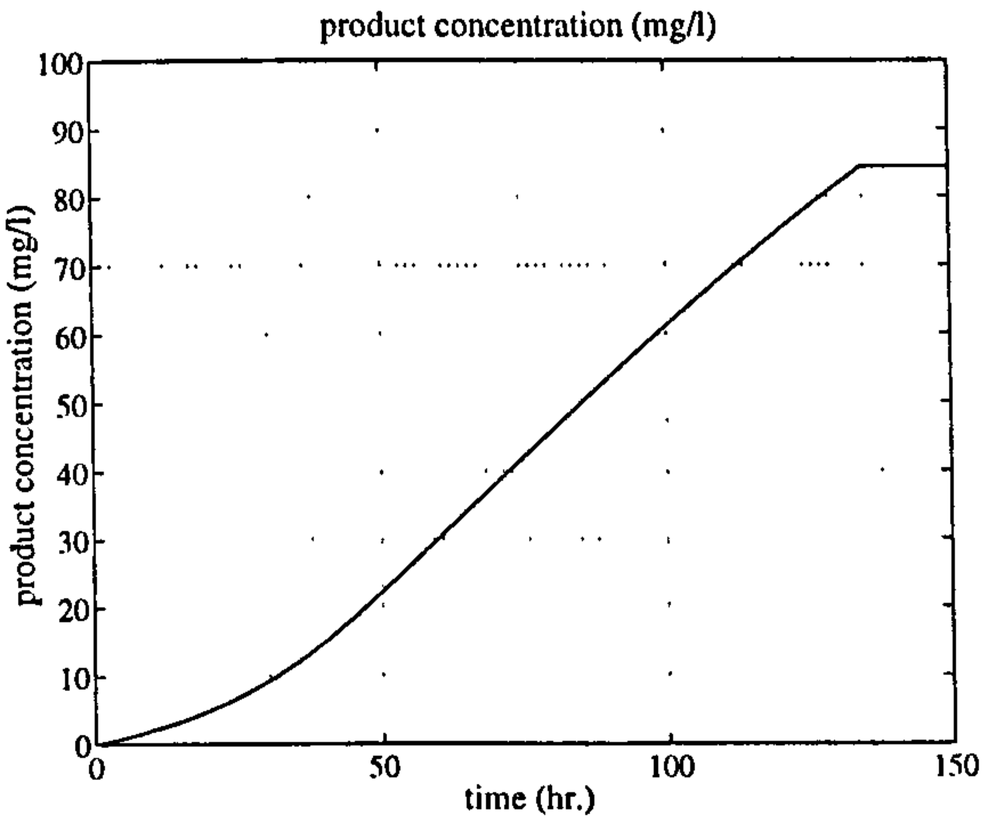

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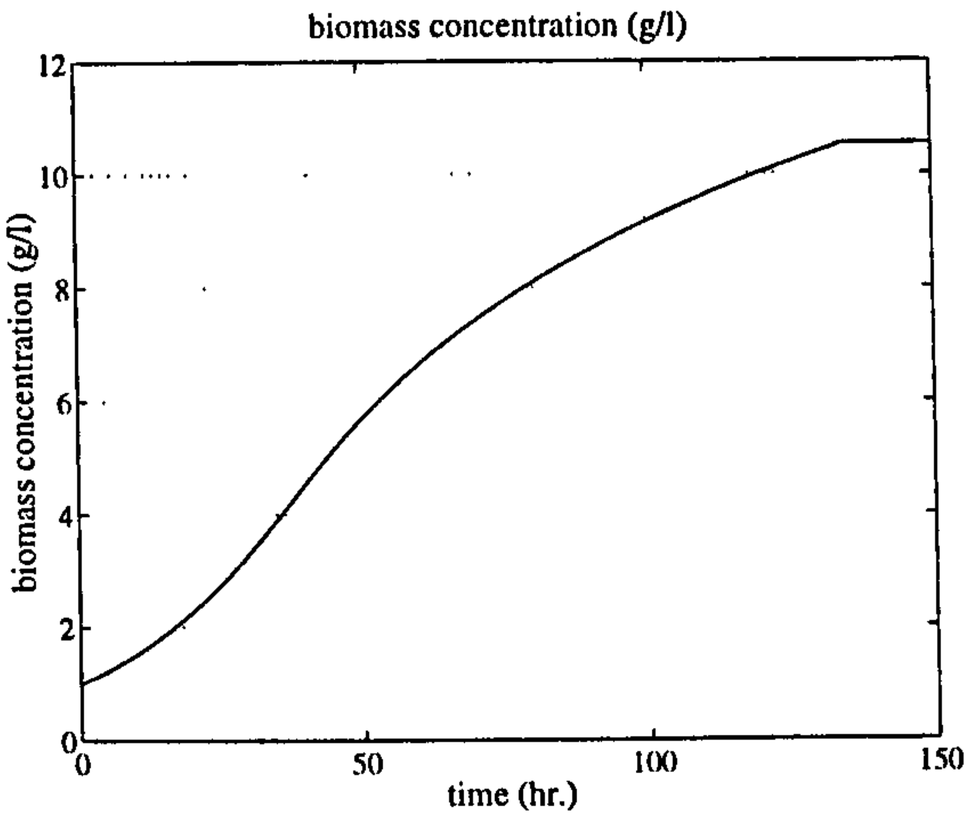

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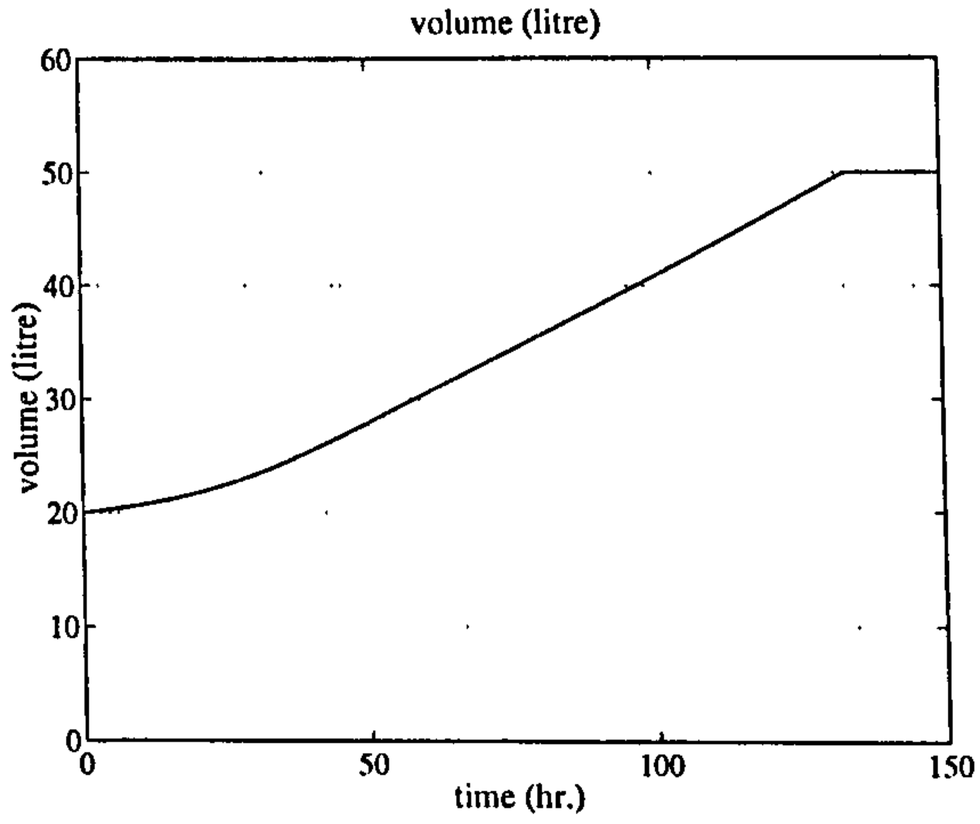
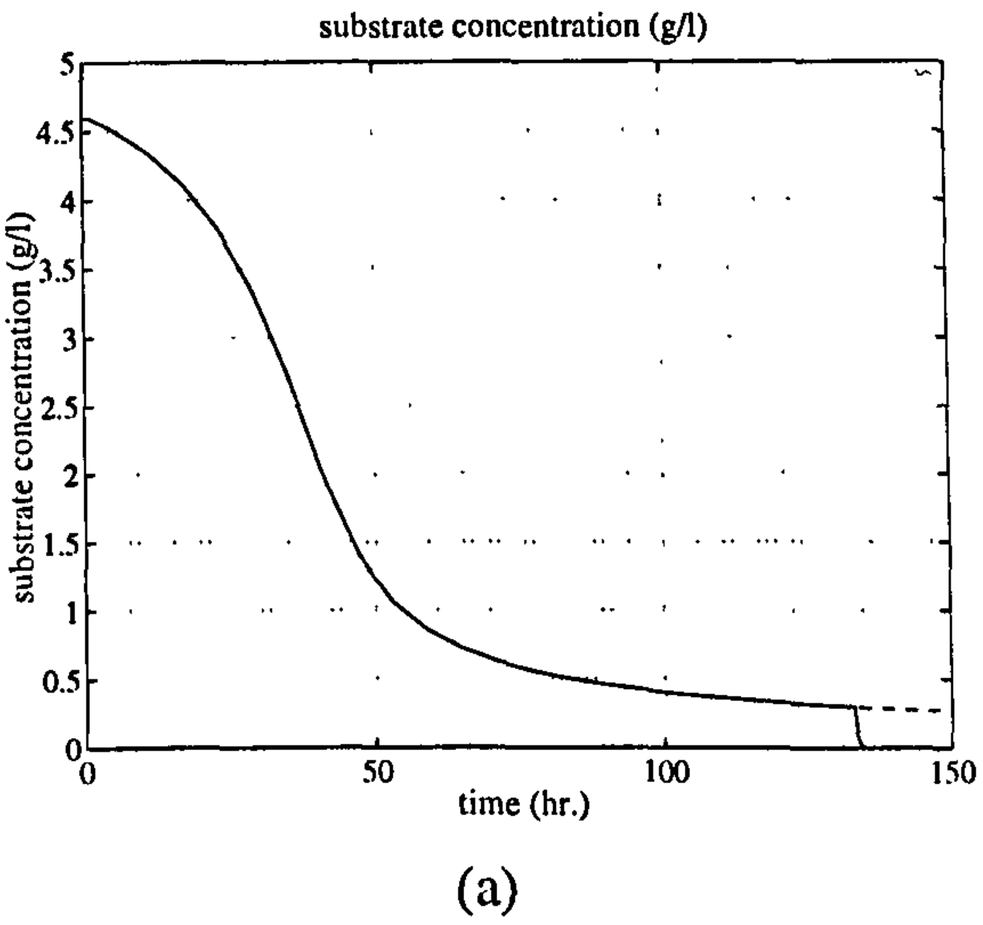
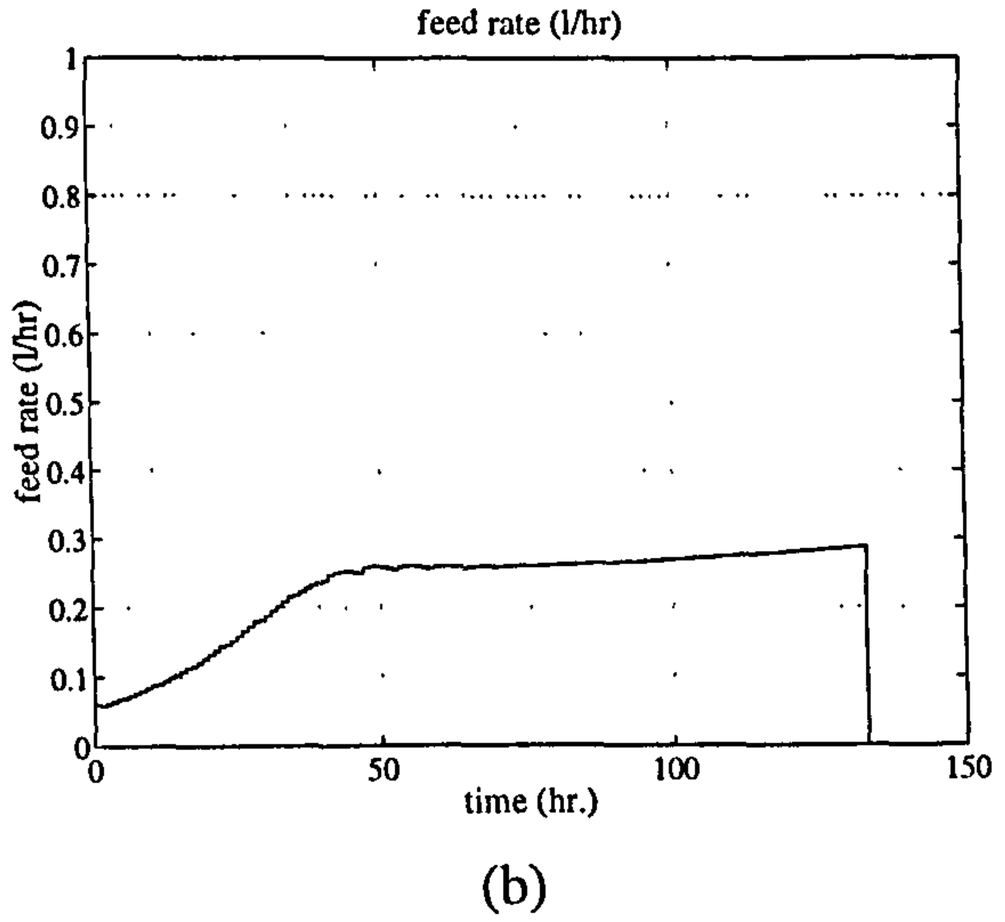


(e)

Figure 5-15 Simulation results of a secondary metabolite production for the OLOFP method (in perfect model)



(a)



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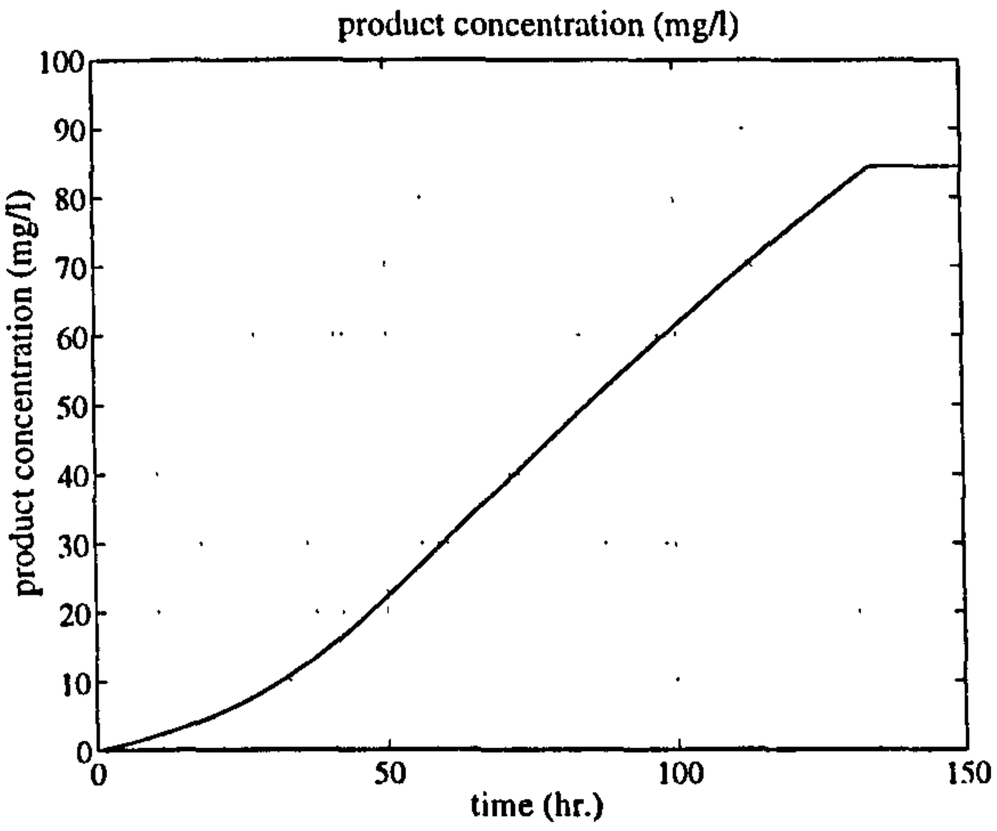

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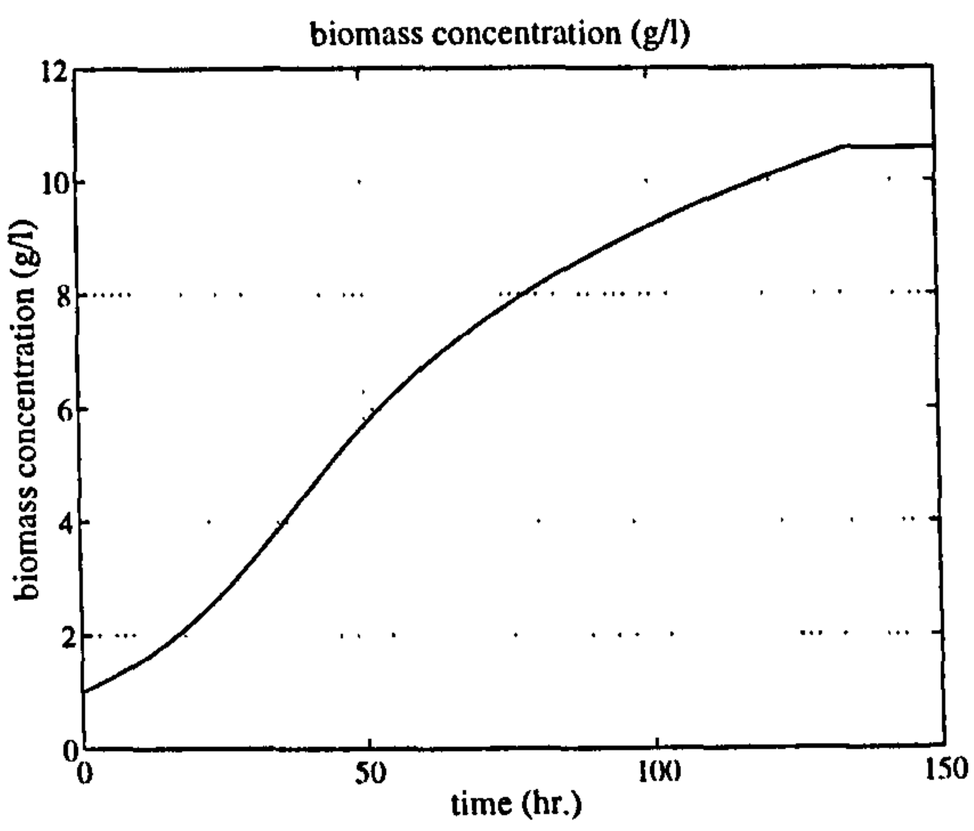

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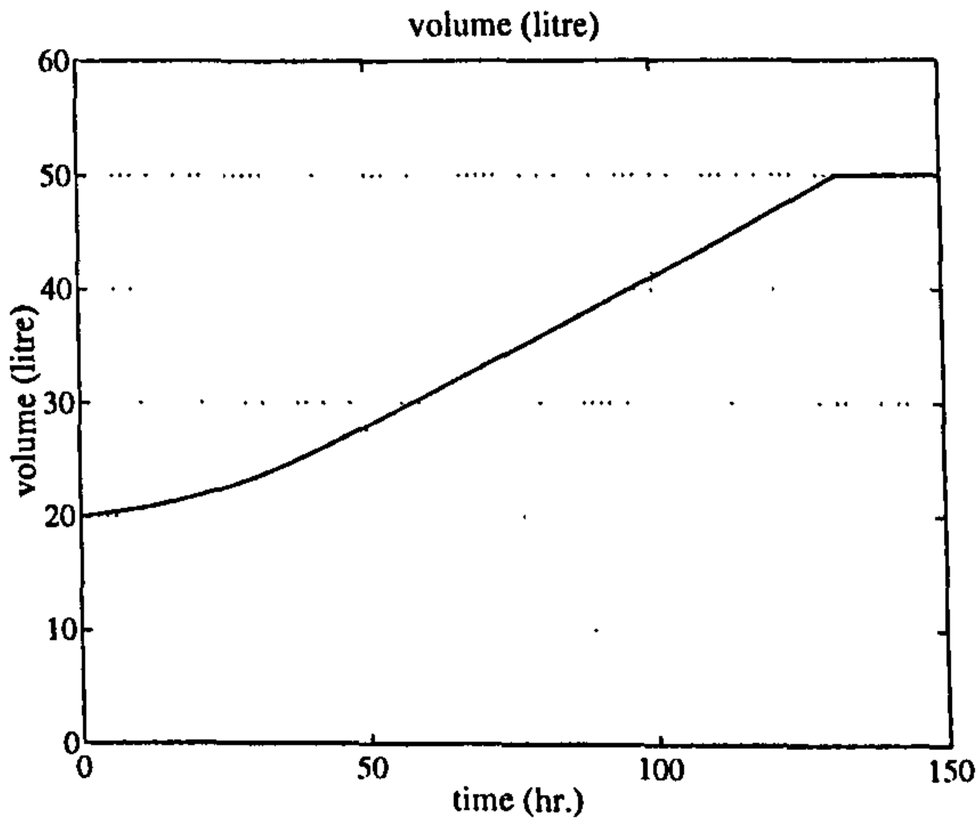


(e)

Figure 5-16 Simulation results of a secondary metabolite production for the OLOFP method (in perfect model)

dashed line in (a): an optimal substrate concentration profile

5.2.2.2 Control simulation with model/process mismatch

We are now considering the case where the obtained model does not fully correctly represent the process. As used in the primary metabolite production in Section 5.2.1.2, we assumed a small error in parameter Y_{xs}, which can be seen as the conversion rate of biomass obtained from a unit of substrate. The process parameter is still at 0.164. The variation for Y_{xs} is assumed to be plus and minus 10 % of the correct value (0.149 and 0.182). Since the nonlinear state feedback equation for the OLOFP method is also available for this process (refer to Equation (5-16)), we will compare the CLOC method with two cases in the OLOFP method. These cases are purely open loop feed rate determination and feed rate with feedback calculated from Equation (5-16). The results from the previous section on the perfect model case will be used as standard for comparing the performance of the OLOFP and CLOC methods as they provided the optimal solutions.

The simulation results for the plant/model mismatch are shown in Figure 5-17 to Figure 5-22. The length of process operating time, the maximum product and biomass obtained are tabulated in Table 5-5 and Table 5-6 for the CLOC and OLOFP methods.

The OLOFP method in which feed rate is purely open loop pre-determined is considered first. The simulation results are shown in Figure 5-17 for the model parameter Y_{xs} smaller than the process and in Figure 5-18 for the model parameter Y_{xs} higher than the process. For a smaller parameter value (Y_{xs} = 0.149), the process is fed with higher substrate feed rate than it really needs. This results in the substrate concentration in the reactor increases higher than the level it should be. (compare Figure 5-17 (a) with the optimal one in the perfect model case in Figure 5-15 (a)) The process operation time is therefore reduced to 128 hr due to the higher biomass growth rate. However, the maximum product obtained is only around 80 mg/l comparing with 84 mg/l in the perfect model case. For a higher

parameter value (Y_{xs} = 0.182), the process is fed with lower rate than it really needs. This results in the substrate concentration in the reactor decreases lower than the level it should be. (also compare Figure 5-18 (a) with Figure 5-15 (a)) This results in slower growth rate hence the operation time lasts longer at 150 hours The maximum product obtained is however higher than the optimal and at around 89 mg/l. Although the maximum product is higher, the cost factor of the operating time in the objective function is violated. The optimal operating time in this process at this cost factor is 134 hr. The shorter time in the previous case (smaller parameter) was also not necessary especially when it was scarified by lower product being obtained.

The feedback version of the OLOFP method is then considered. The simulation results are shown in Figure 5-19 and Figure 5-20. The singular feed rate is determined by Equation (5-16). For a smaller parameter value case, the determined feed rate is higher than necessary. This results in an increasing in substrate concentration. With the substrate concentration increase, feed rate also increases and stops when the reactor is full. The substrate concentration then decreases. This results in the high growth rate and shorter operating time. The maximum product obtained is only 33.5 mg/l. For a higher parameter value case, the calculated feed rate is lower than the process needs. This results in the substrate concentration level lower than the optimal one (compare Figure 5-20 (a) and Figure 5-15 (a)). The substrate concentration eventually reduces to zero. As the substrate concentration becomes zero, the calculated singular feed rate then becomes zero and the process is stopped without fully filling up the reactor volume (see Figure 5-20 (e)).

For the CLOC method, The simulation results are shown in Figure 5-21 and Figure 5-22. It shows that the optimal substrate concentration profile is followed quite well in both higher and smaller incorrect parameter value. The patterns are similar to the optimal ones shown

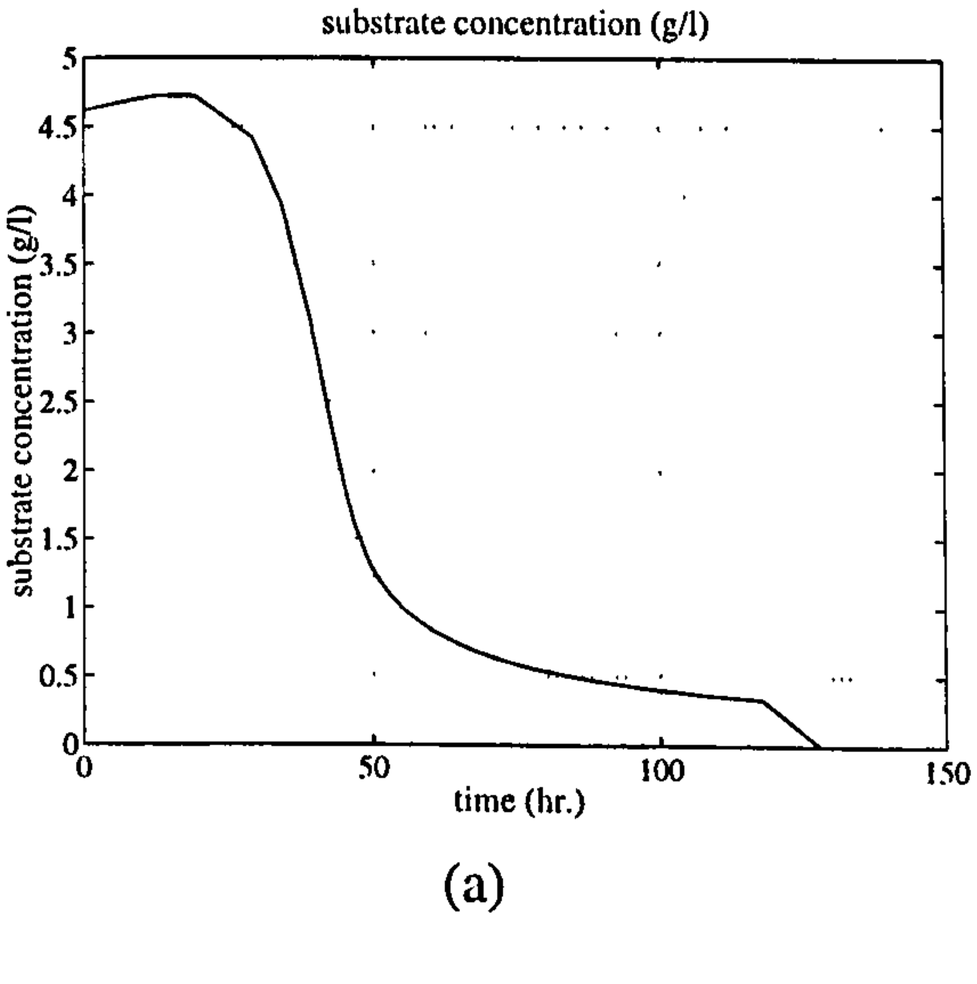
in Figure 5-15 and Figure 5-16. The operating time lasts for 137 hr for the smaller parameter case and 138 hr for the higher parameter case (comparing with 134 hr for the optimal one with correct parameter). In both cases, the maximum products obtained are around 86 mg/l which are higher than the one obtained in the correct parameter case. This is due to the longer operating time than the optimal in both cases.

Table 5-5 CLOC method in process/model mismatch

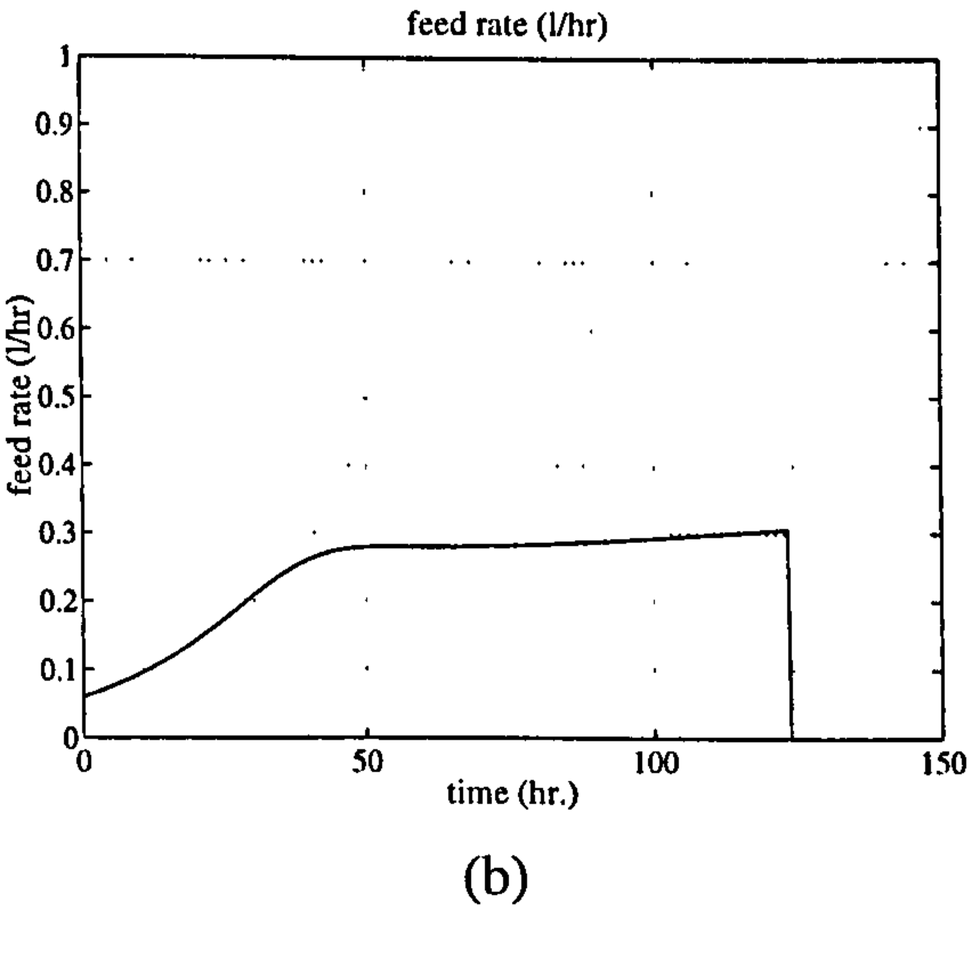
Control Method	CLOC	
Parameter variation in Y_{xs}	+ 10 %	- 10 %
Finish time (hr.)	138	137
Maximum product (mg/l)	86.32	85.99
Maximum biomass (mg/l)	10.54	10.54

Table 5-6 OLOFP method in process/model mismatch

OLOFP method	Pre-determined feed		Using feedback	
Parameter variation in Y_{xs}	+ 10 %	- 10 %	+ 10 %	- 10 %
Finish time (hr.)	150	128	100	82.5
Maximum product (mg/l)	89.14	79.85	23.07	33.52
Maximum biomass (mg/l)	10.54	10.54	4.13	10.54



(a)



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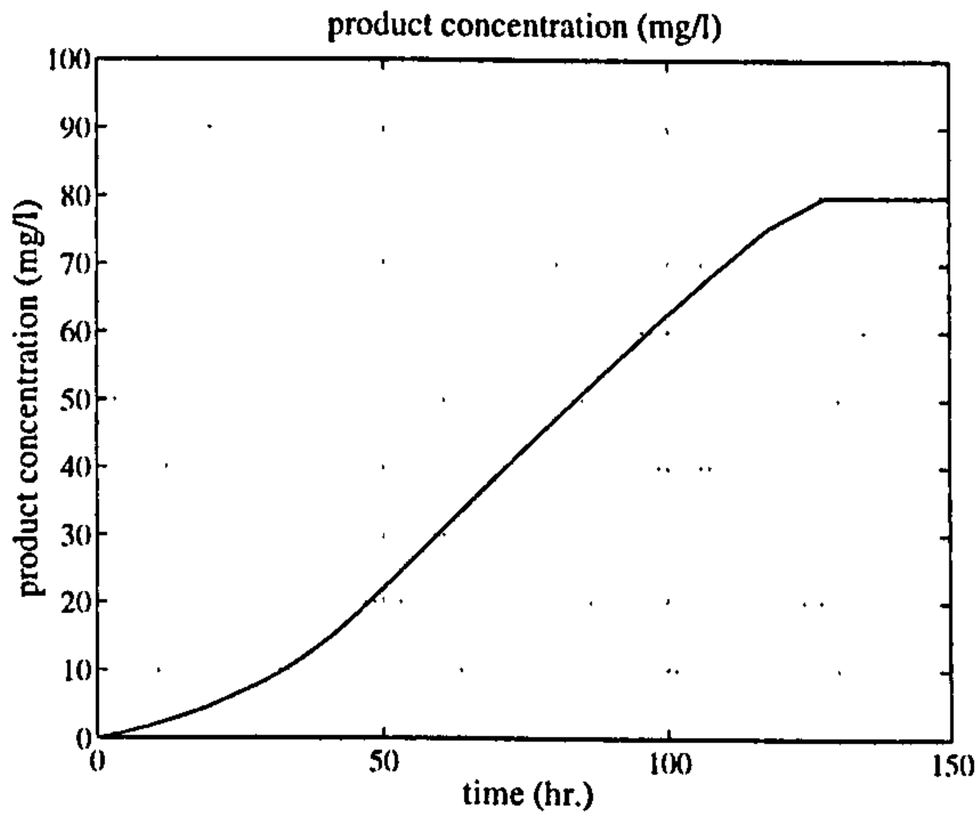

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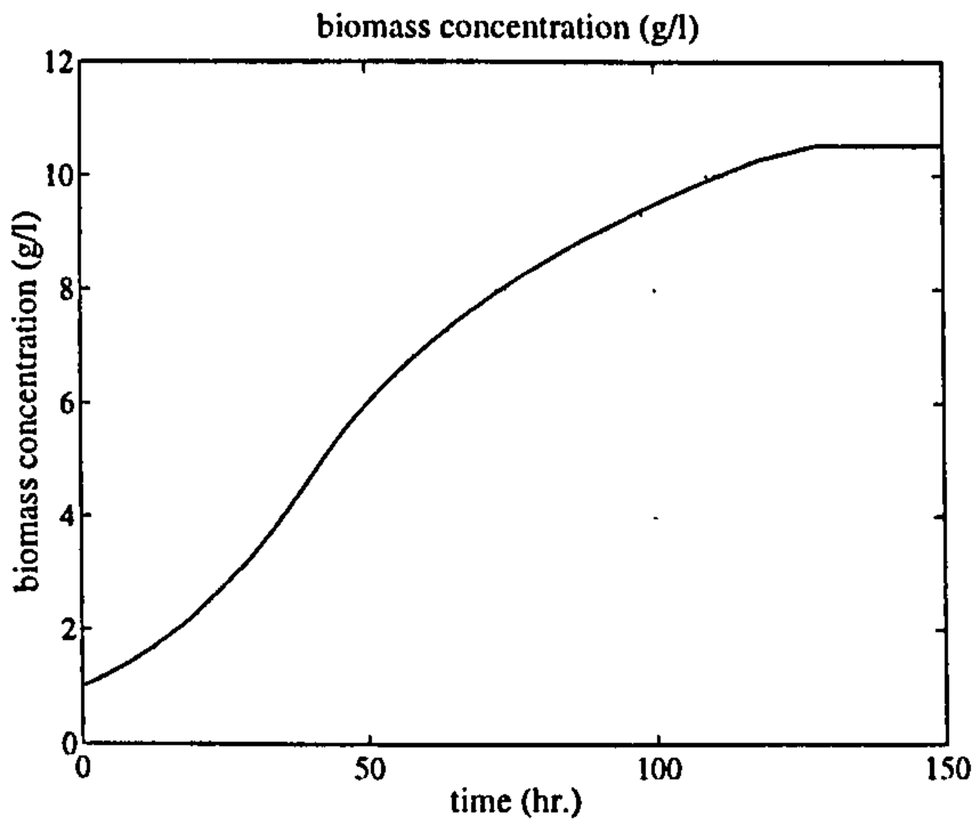

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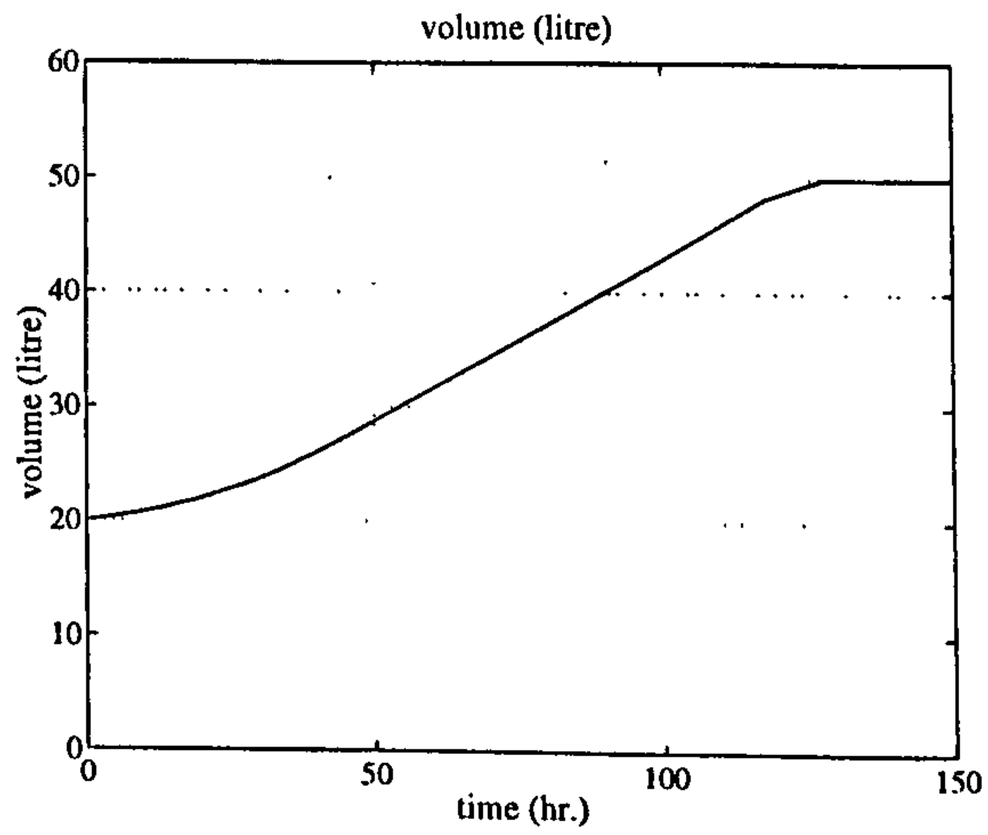
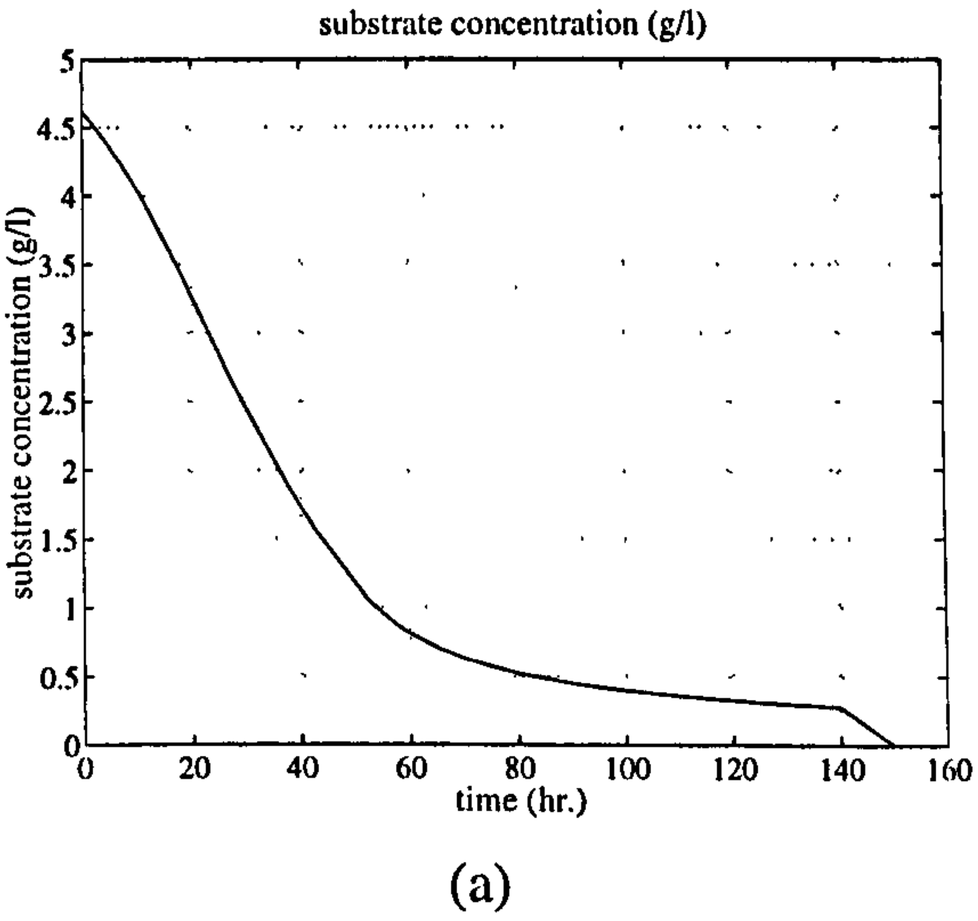
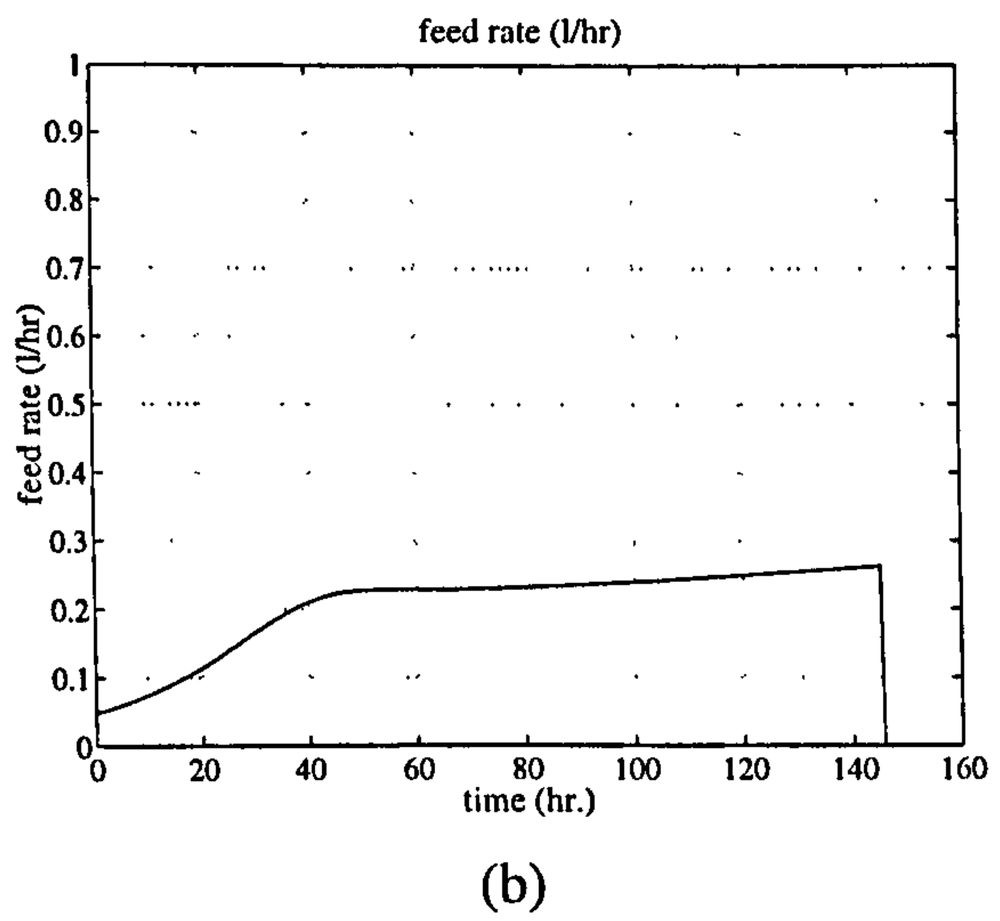


(e)

Figure 5-17 Simulation results for the OLOFP method using pre-determined feed rate (model parameter is smaller than the plant parameter) - a secondary metabolite production.



(a)



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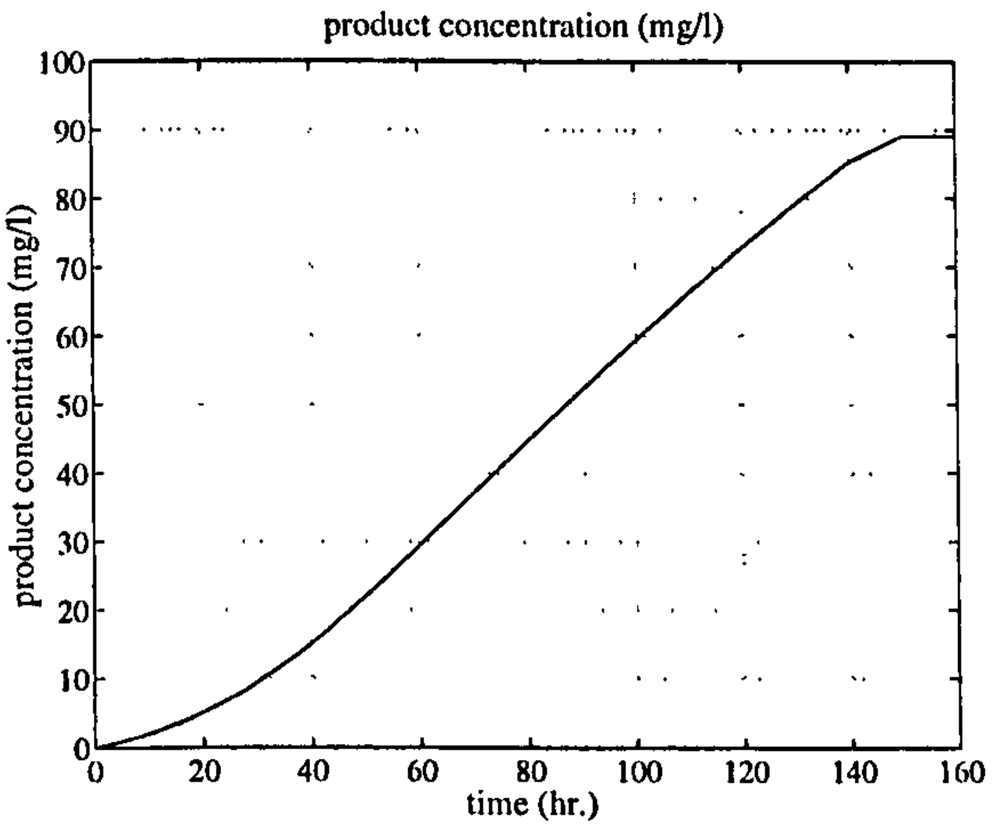

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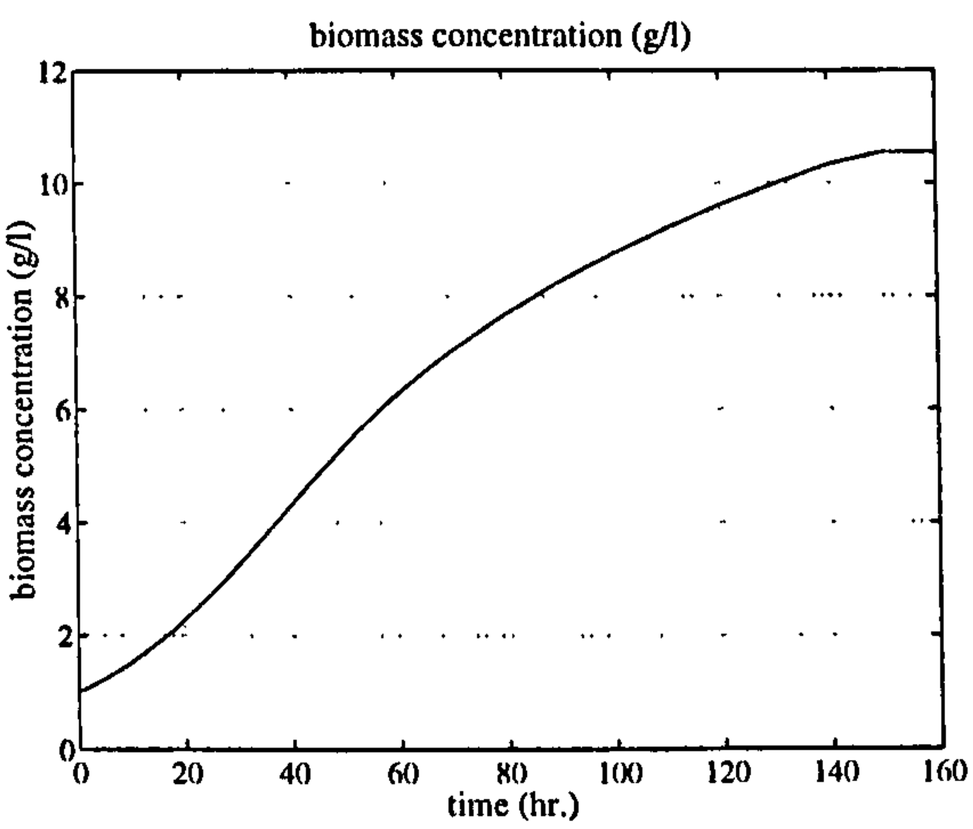

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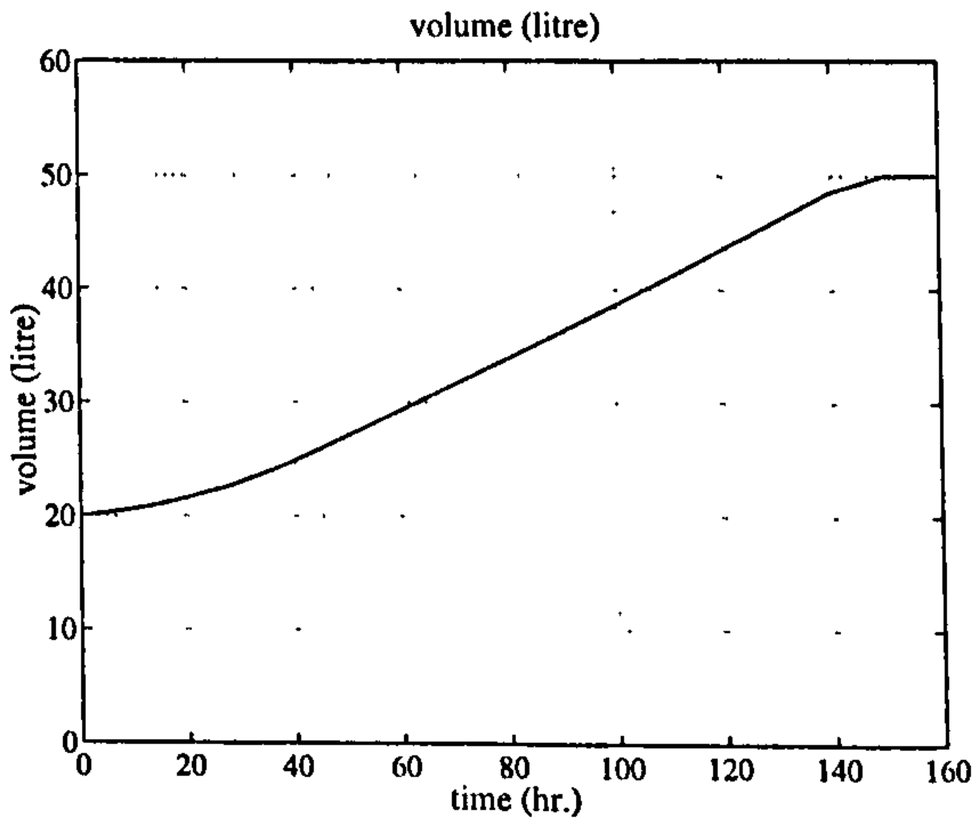

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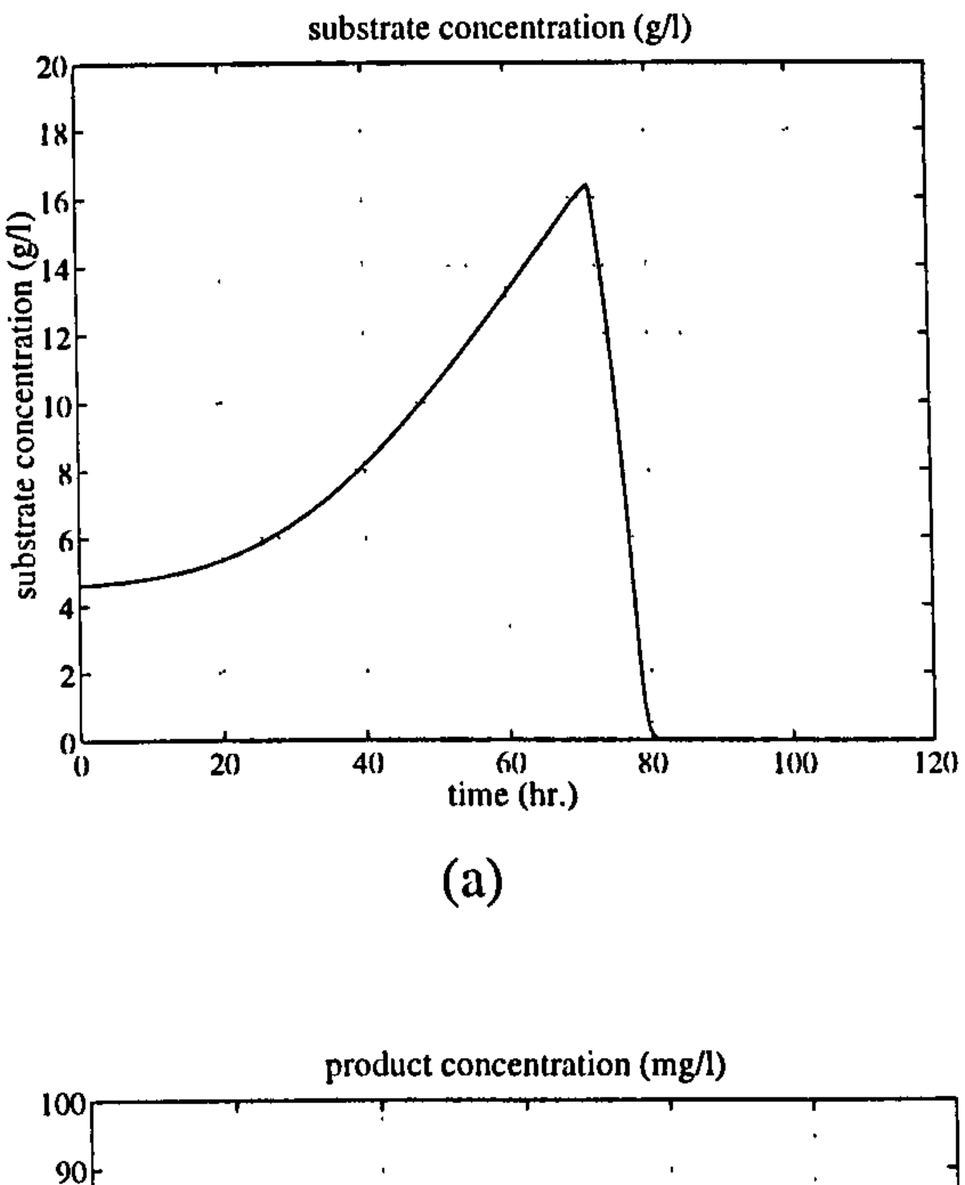
Figure 5-18 Simulation results for the OLOFP method using pre-determined feed rate (model parameter is higher than the plant parameter) - a secondary metabolite production.



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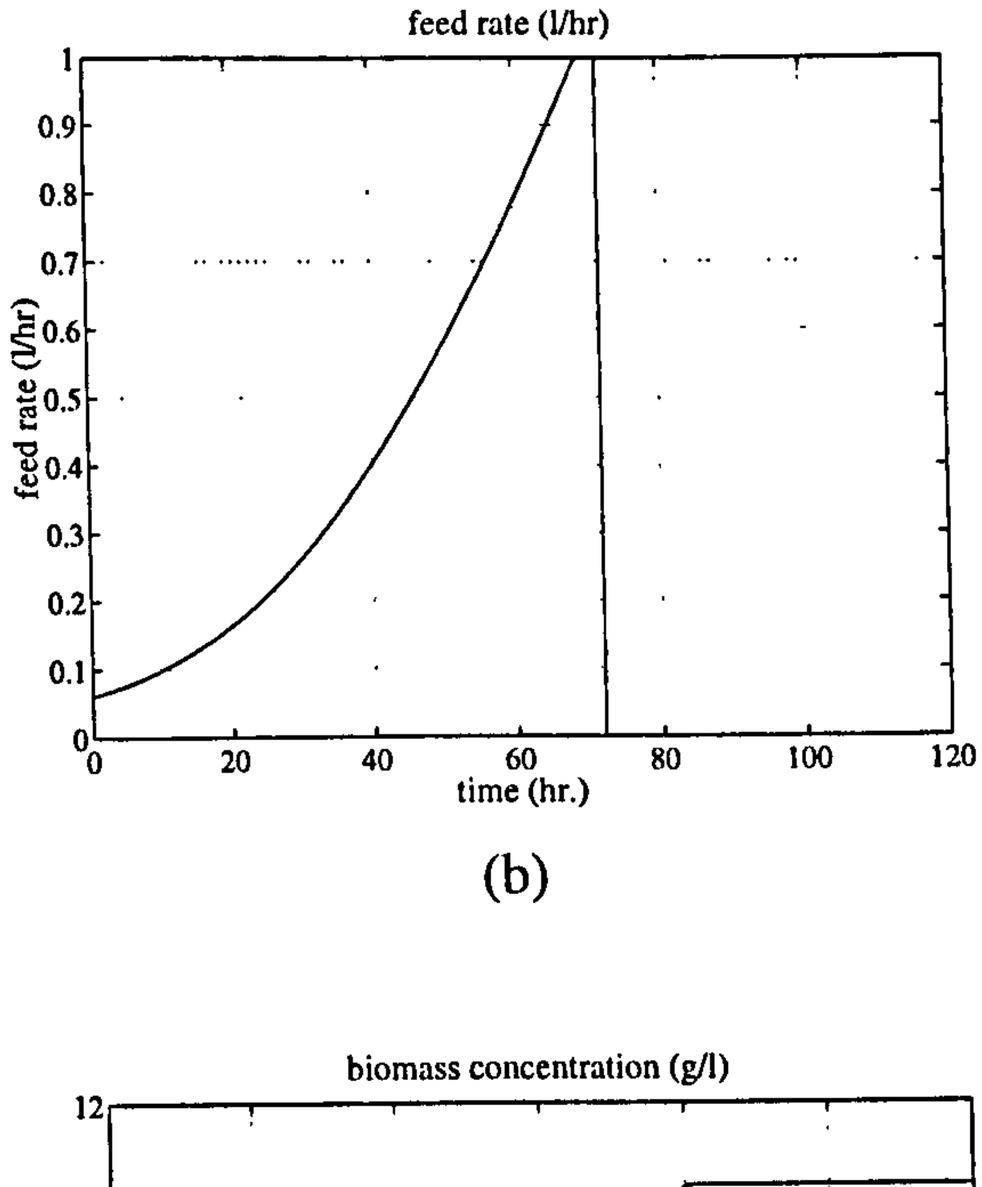

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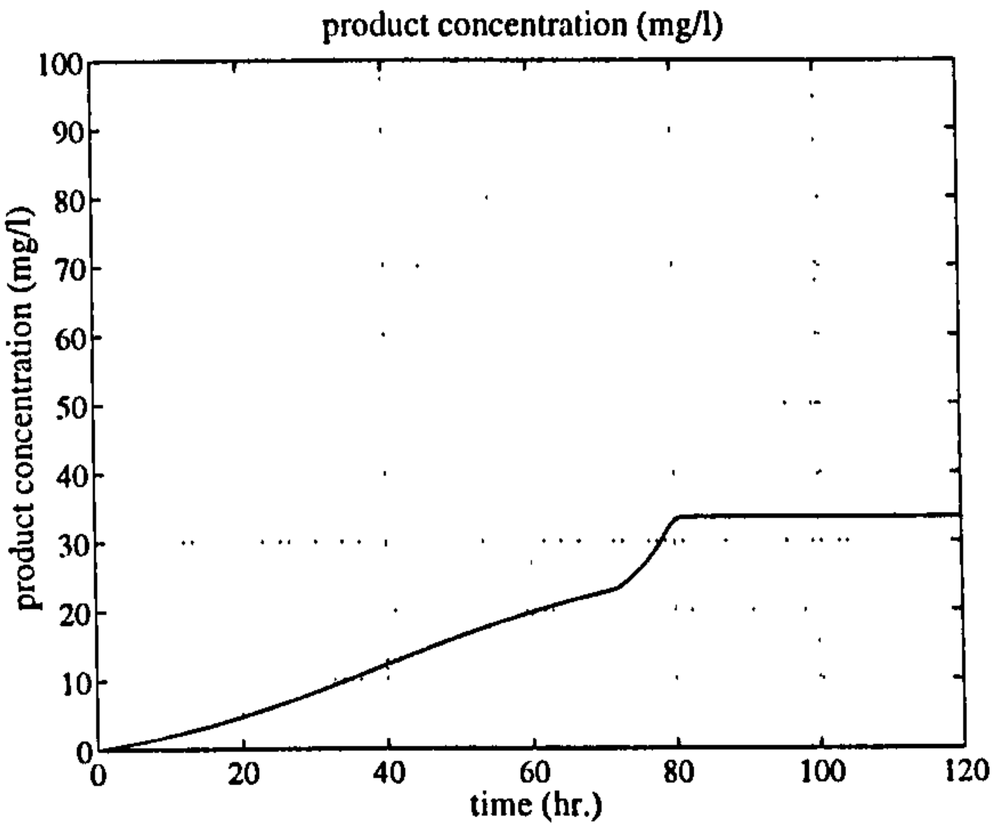

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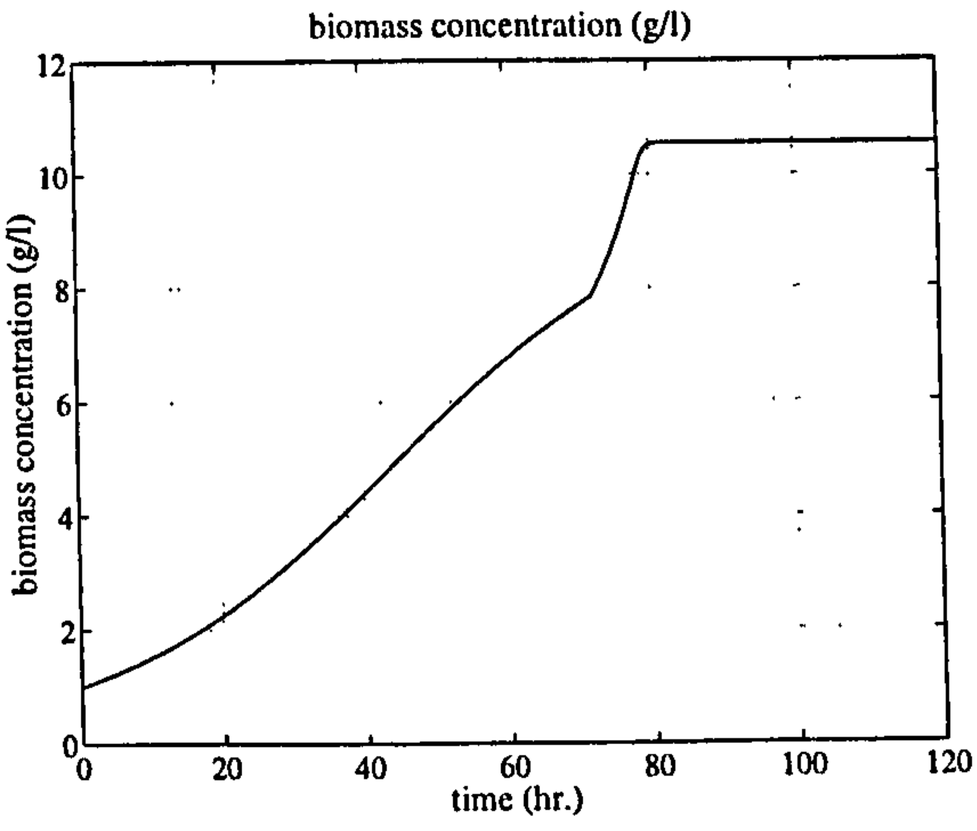

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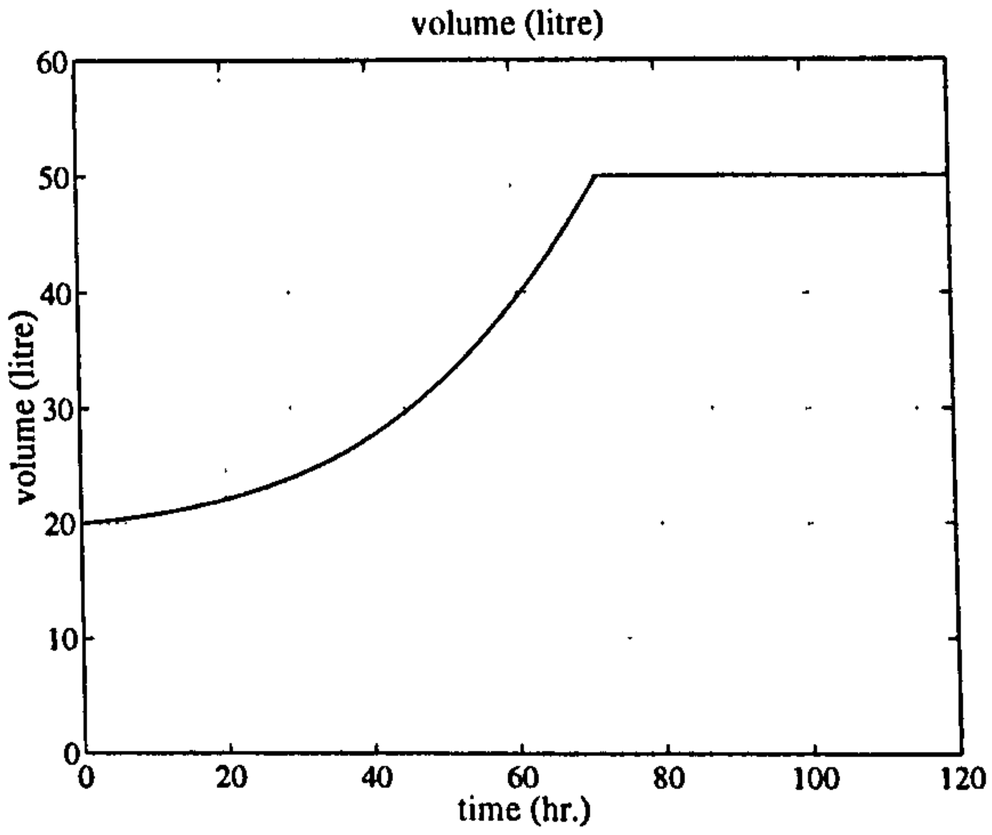
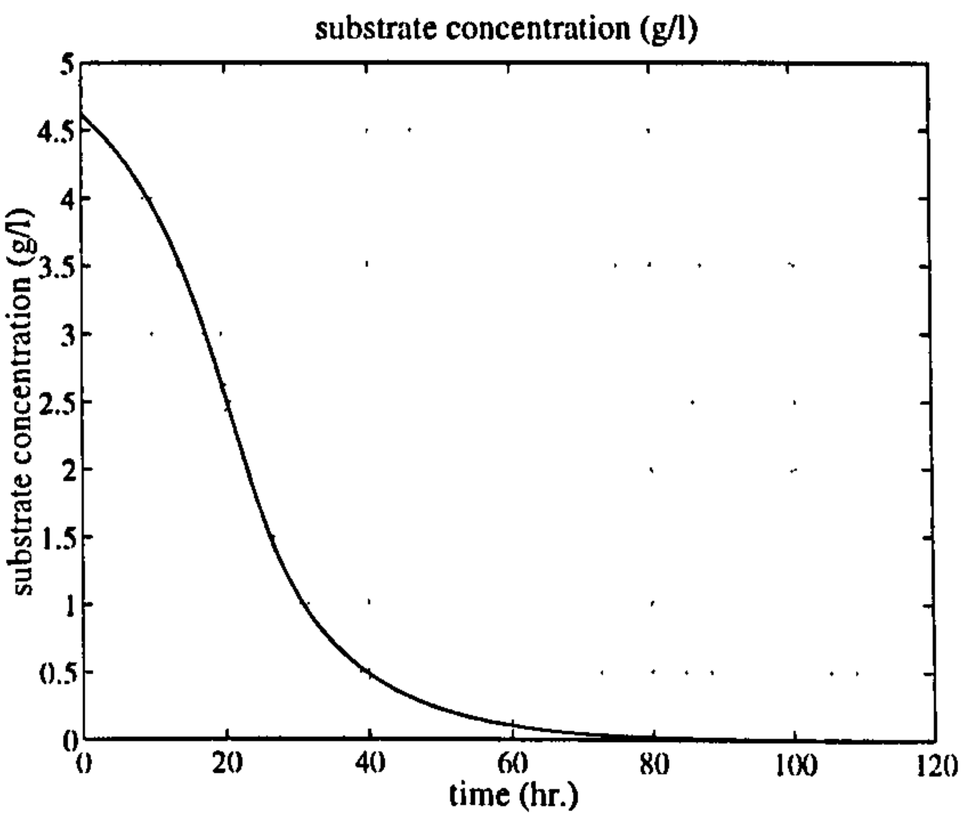


(e)

Figure 5-19 Simulation results for the OLOFP method using (5-16) for calculating feed rate (model parameter is smaller than the plant parameter) - a secondary metabolite production.



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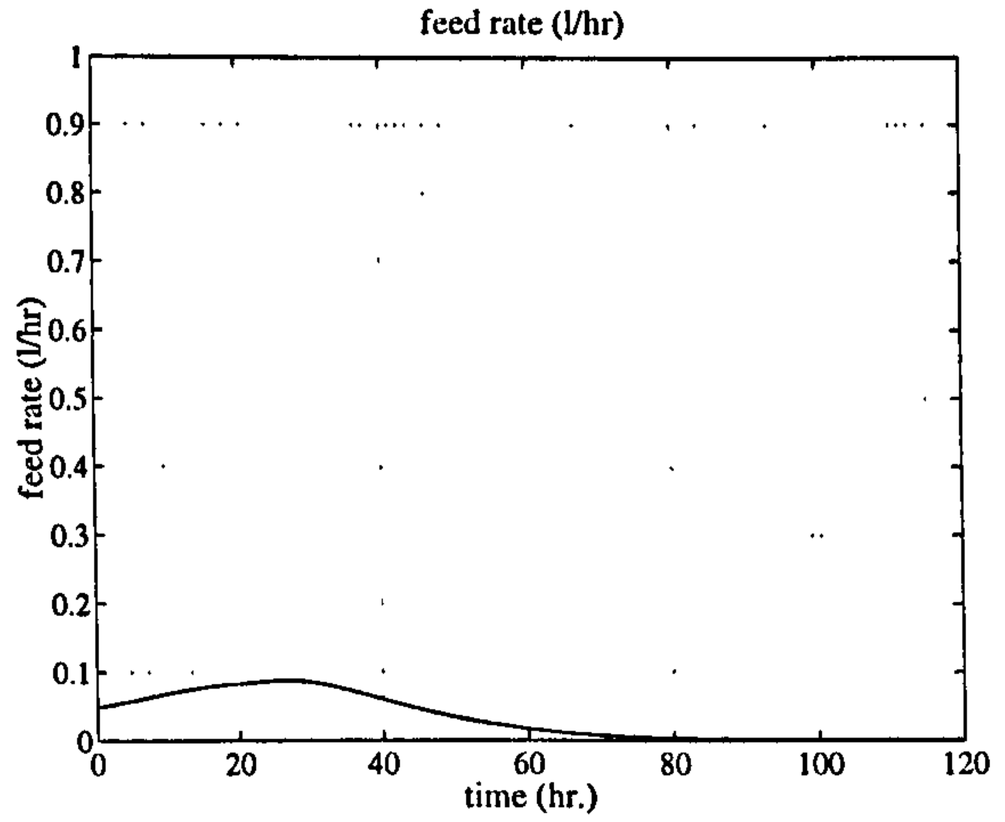

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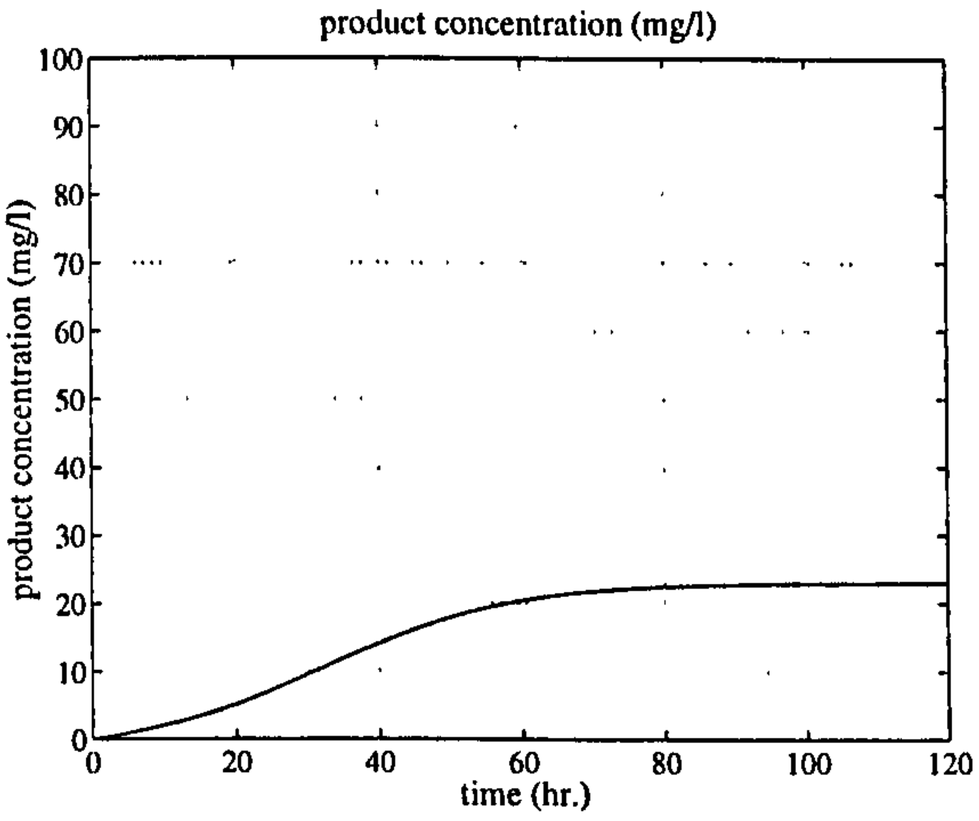

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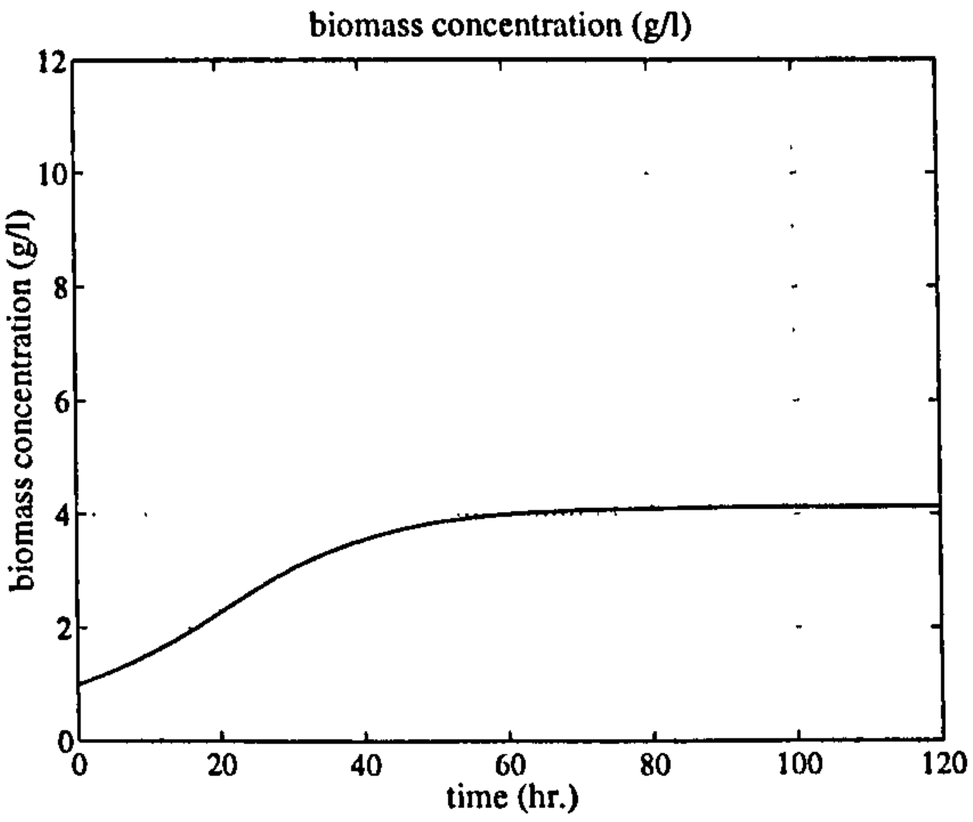

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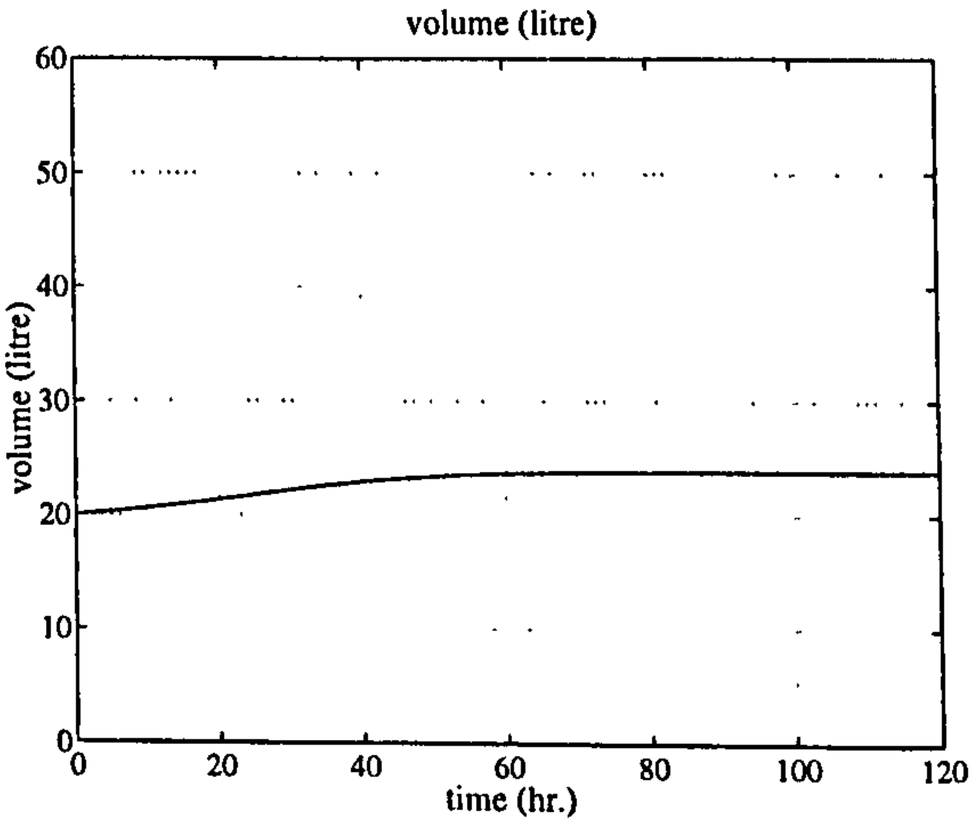
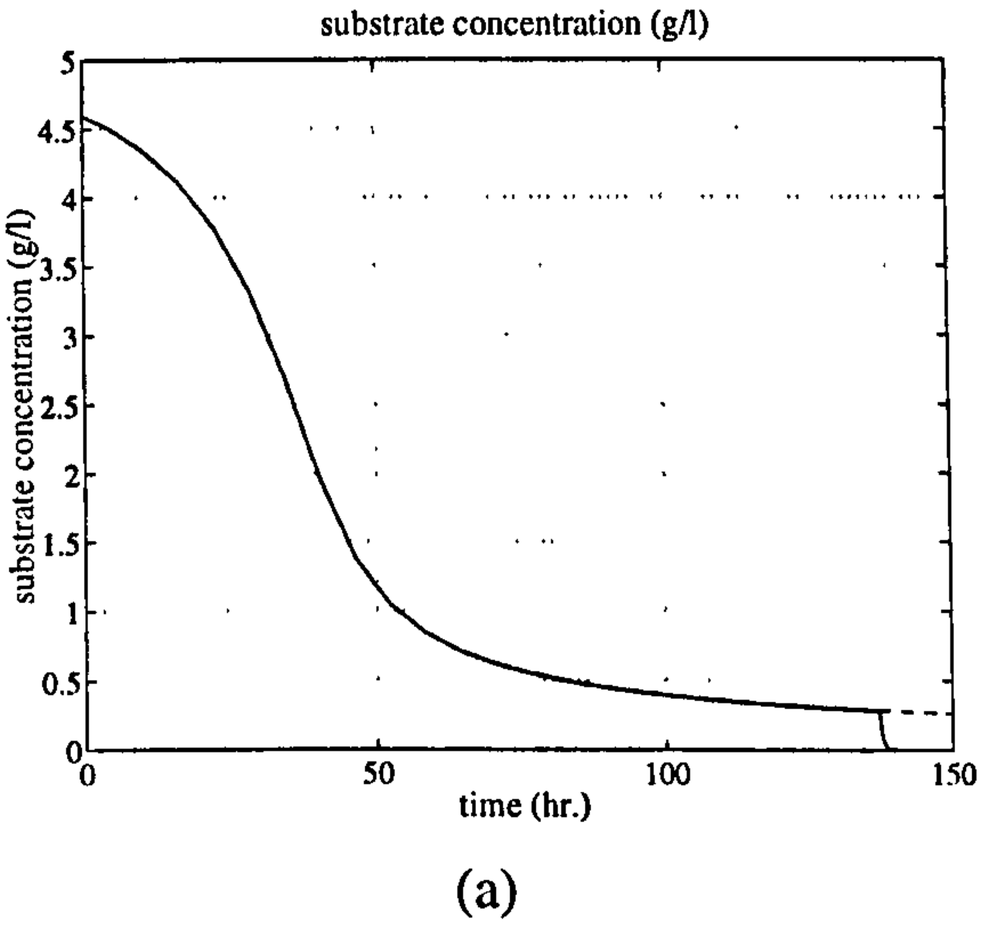


(e)

Figure 5-20 Simulation results for the OLOFP method using (5-16) for calculating feed rate (model parameter is higher than the plant parameter) - a secondary metabolite production.



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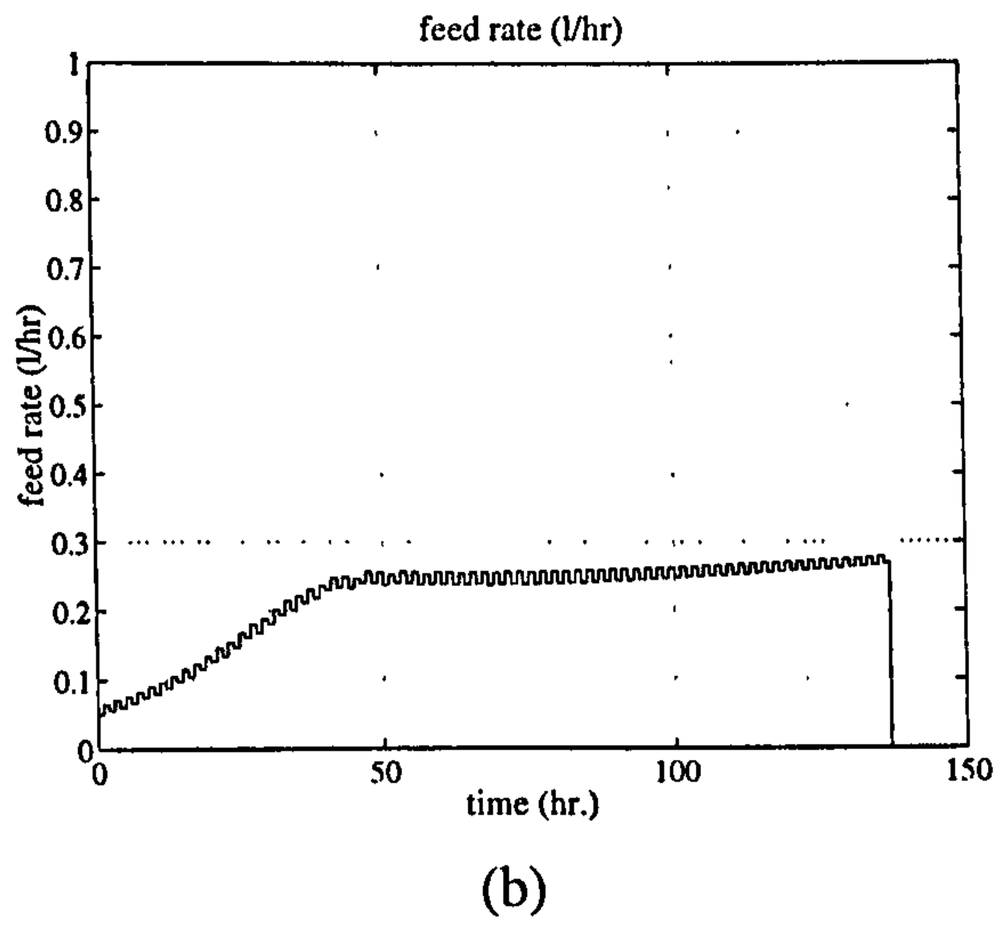

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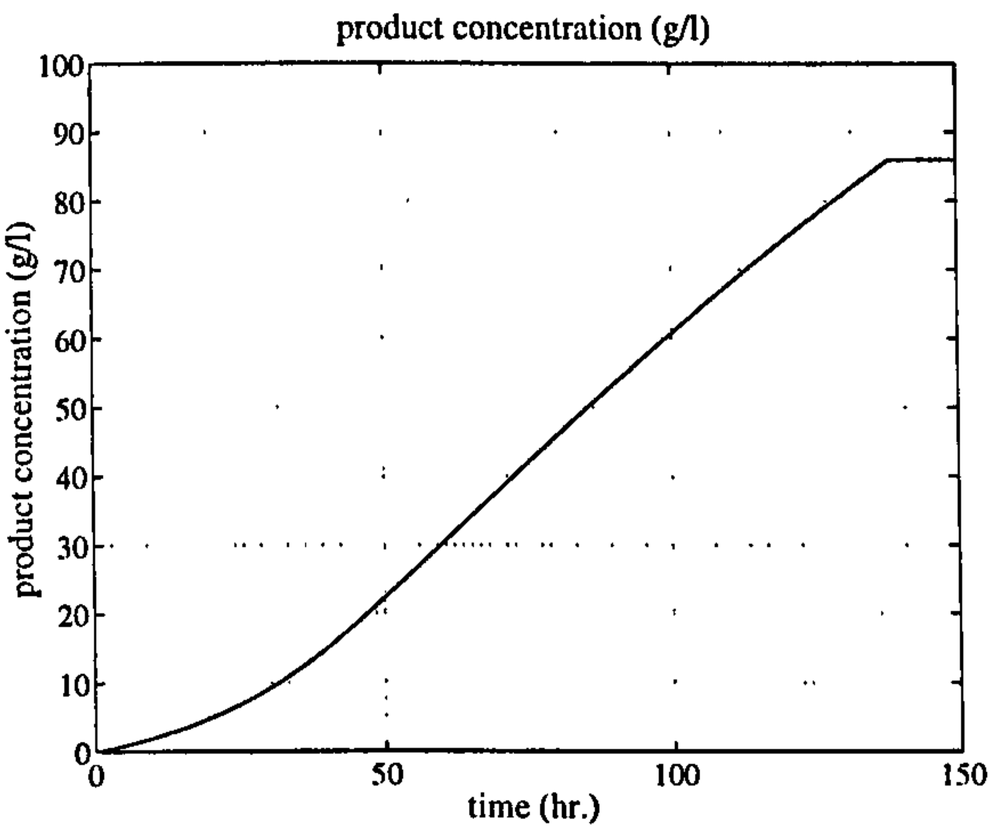

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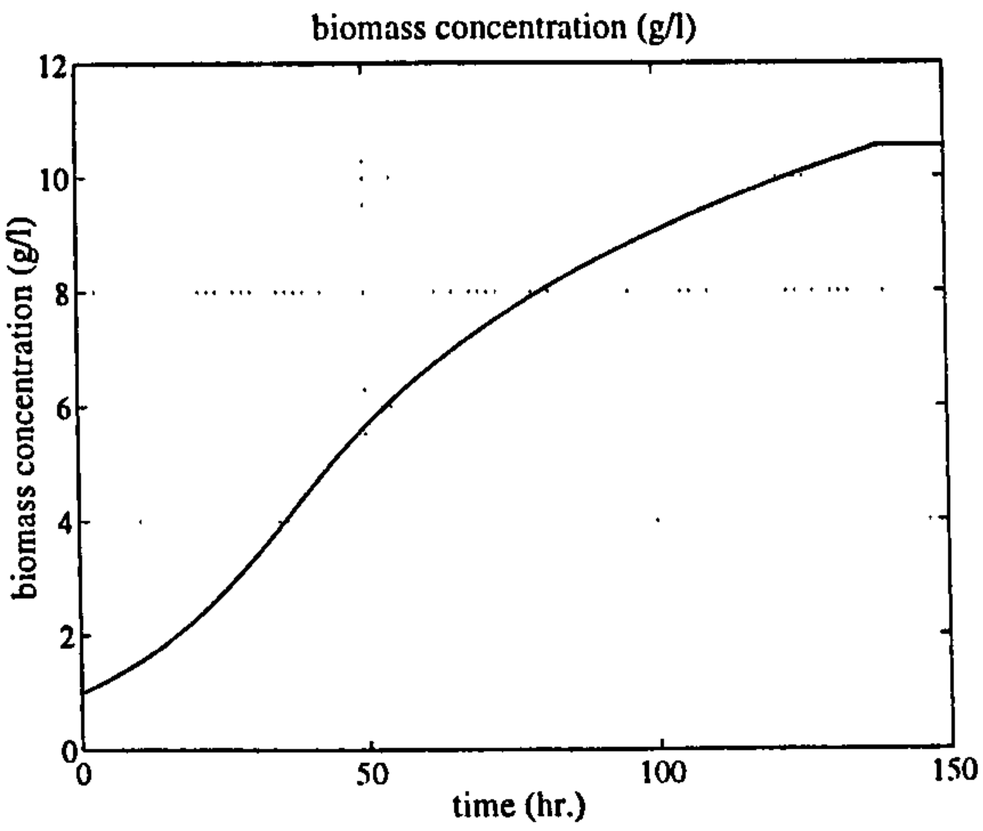

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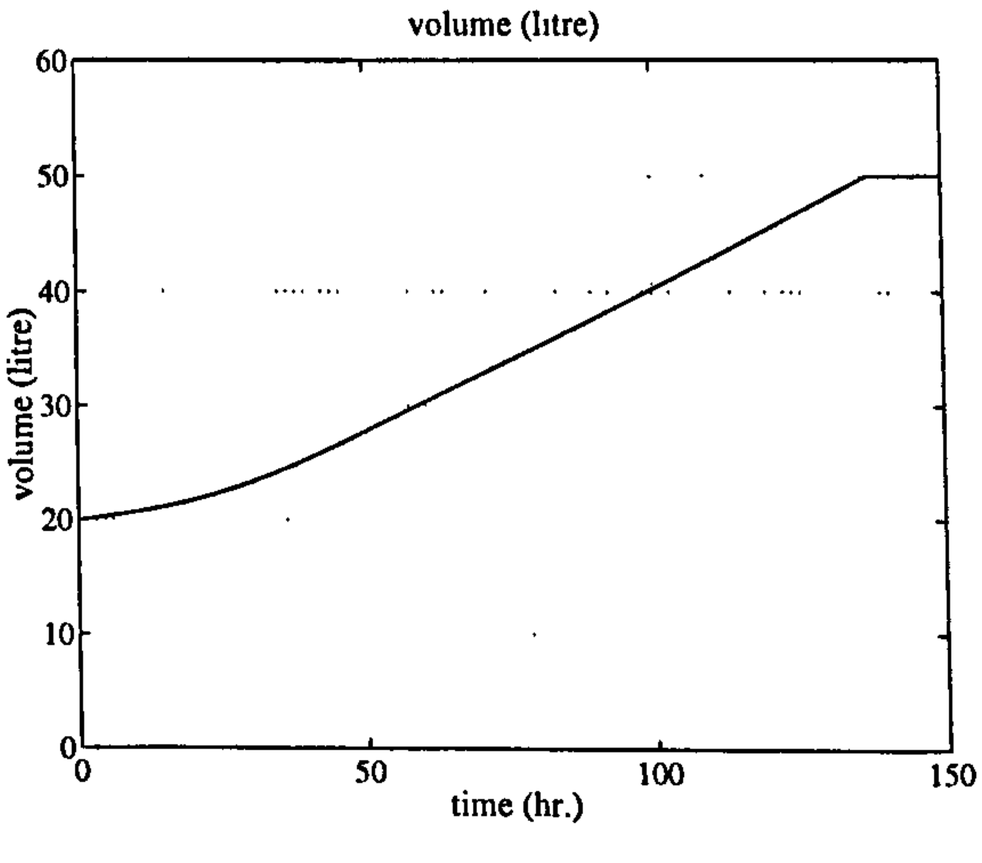
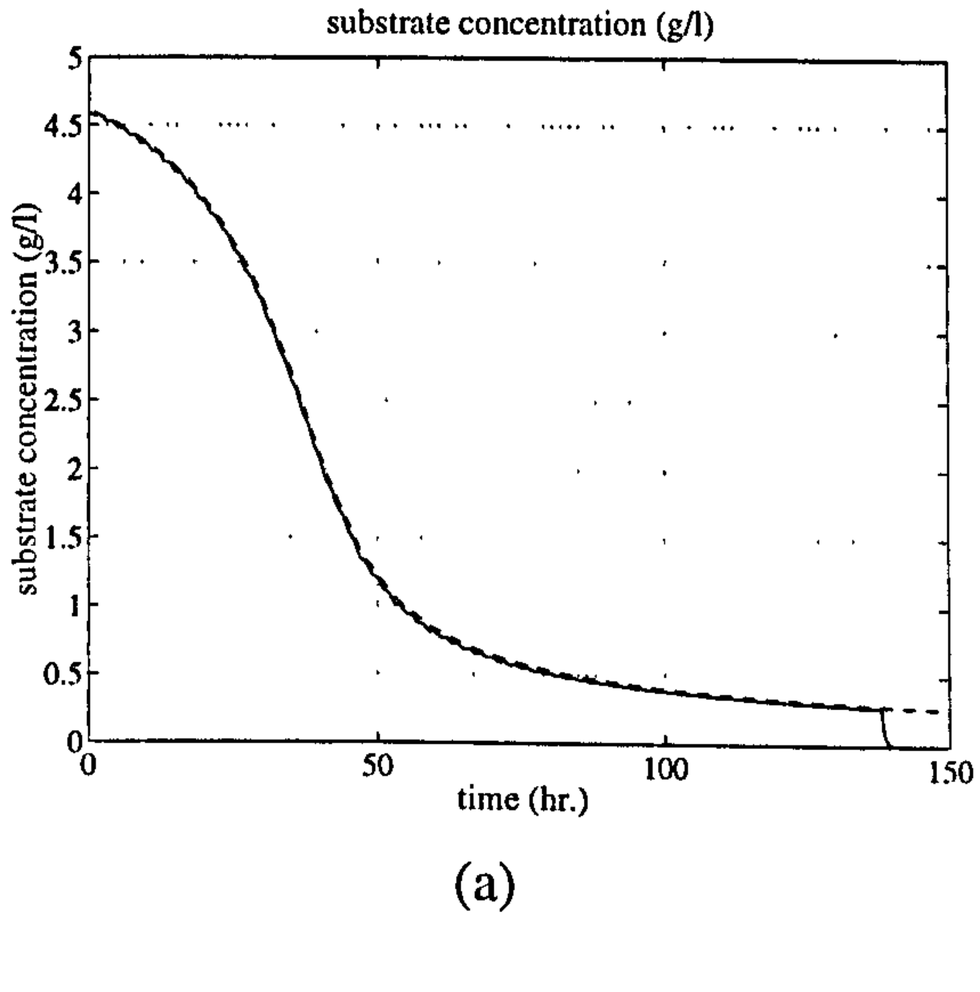


(e)

Figure 5-21 Simulation results for the CLOC method (model parameter is smaller than the plant parameter) - a secondary metabolite production.



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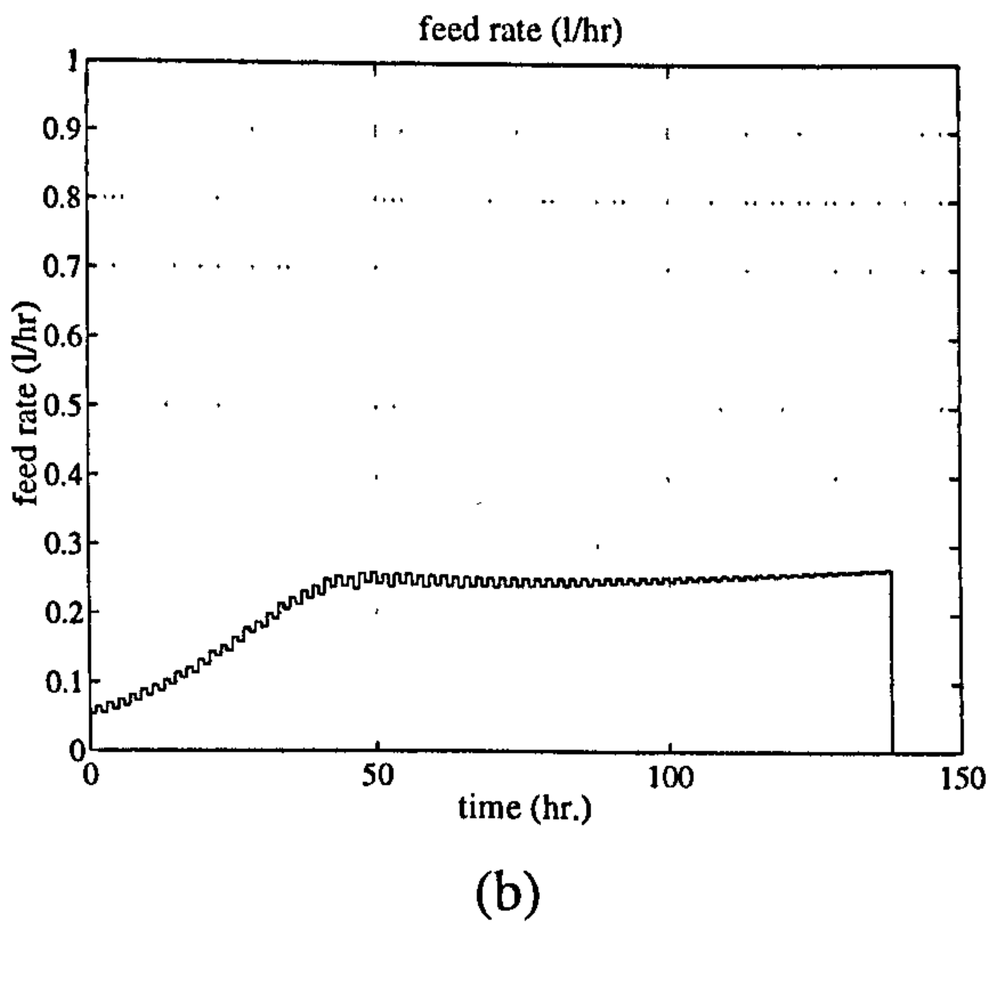

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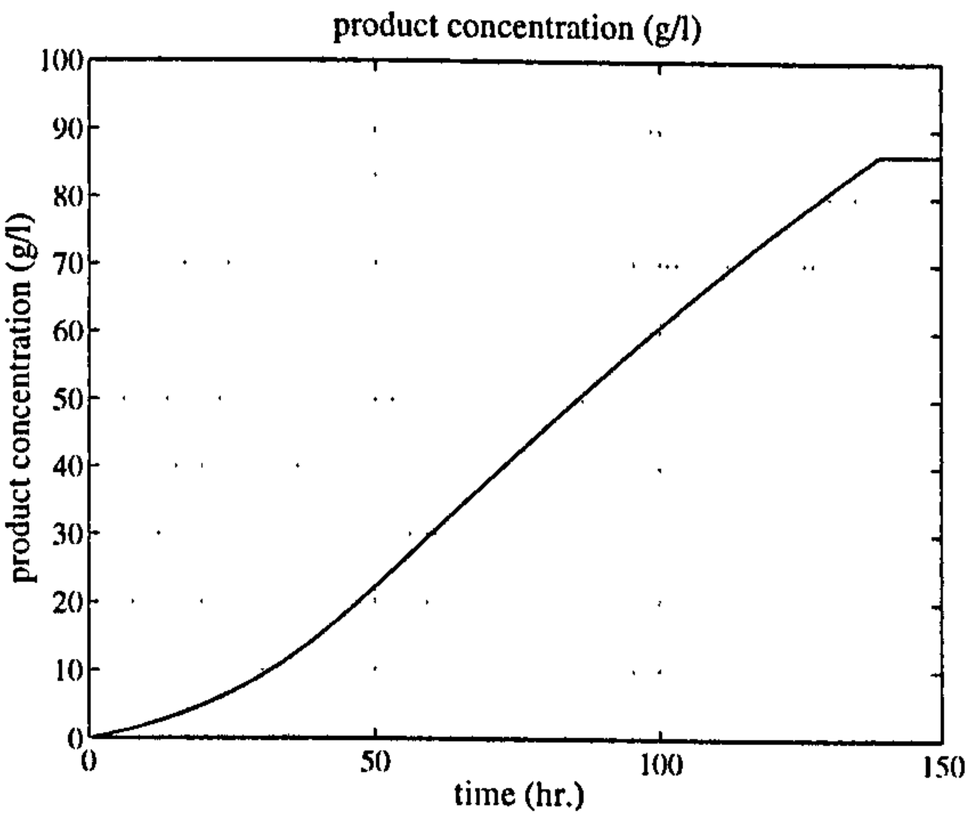

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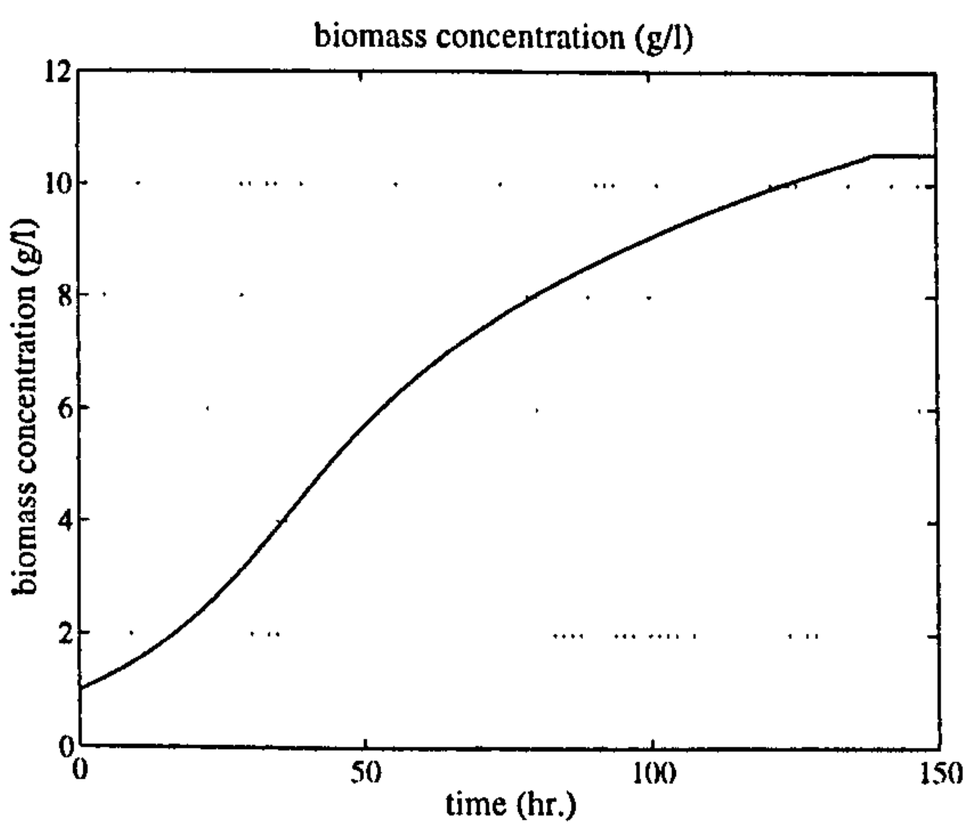

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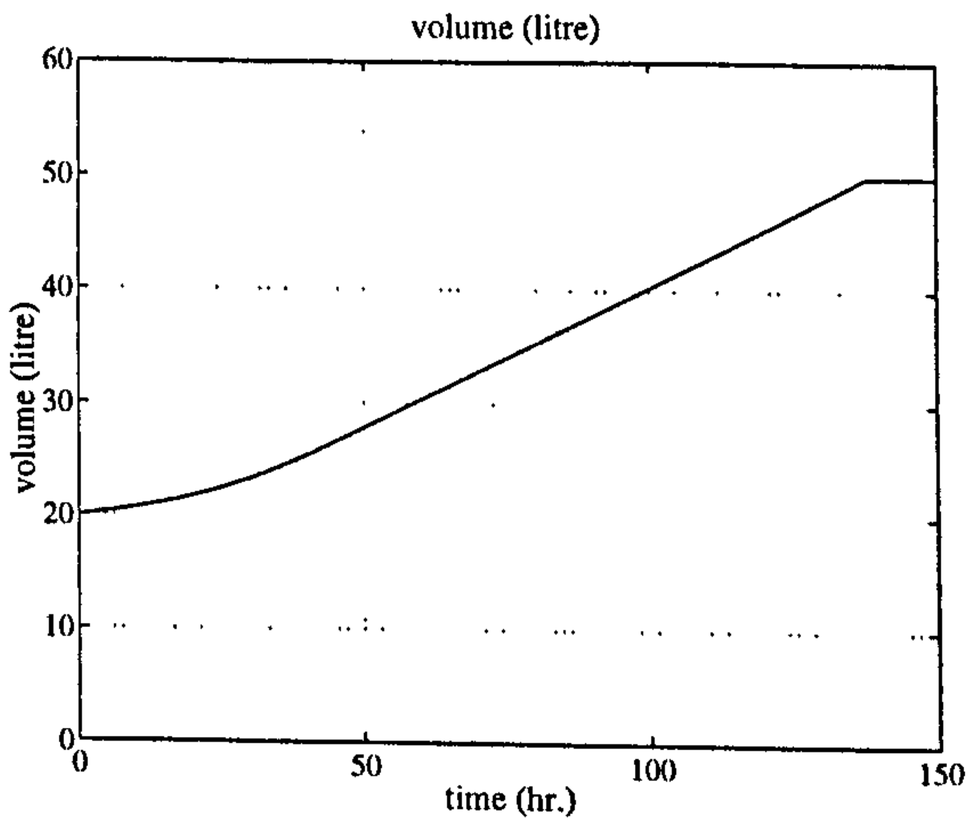


(e)

Figure 5-22 Simulation results for the CLOC method (model parameter is higher than the plant parameter) - a secondary metabolite production.

The control objective is to maintain the process as close as possible to the optimal one. The comparison of both methods can therefore be shown by the deviation of operating time and maximum product obtained from the optimal cases in which the correct model parameter is used. The results are shown in Table 5-7 and the following formulations are used in the calculation:

$$\text{deviation in Time (\%)} = \frac{t - 134}{134} * 100$$

$$\text{deviation in Product (\%)} = \frac{P - 84.43}{84.43} * 100$$

Where t is process operating time and P is a maximum product.

The optimal operating time and product obtained from the correct parameter case for the OLOFP method (refer to Table 5-4) are 134 hours and 84.43 mg/l.

Table 5-7 Comparison of performance between OLOFP and CLOC

Control method	OLOFP			CLOC	
	Pre-determined		Feedback		
Parameter variation in Y_{xs}	+ 10 %	- 10 %	- 10 %	+ 10 %	- 10 %
Deviation in time (%)	11.9	-4.5	-38.8	3	2
Deviation in product (%)	5.6	-5.4	-60.3	2.2	1.9

The OLOFP method in the table does not contain the case of feedback with higher parameter value since the process does not complete the operation.

From the table, it can be seen clearly that in the case of plant/model mismatch, the deterioration of performance for the OLOFP method is more severe than the CLOC method. The range in maximum product obtained is varied between -5 % to + 5 % for the OLOFP case comparing with under 3 % for the CLOC case. For the variation in operating time, the OLOFP case varies between -4.5 % to 11.9 % comparing with 3 % for the CLOC case. This demonstrates the better performance of the CLOC method over the OLOFP method.

As the cost factor has an effect on the objective function and results in the relationship between biomass and substrate concentration during the optimal period as shown in Equation (5-15), it will be explained in more detail in the next section.

5.2.2.3 Effect of cost factor on secondary metabolite production

In the previous section, we have shown that, at the specific cost factor, the CLOC method performed equally well as the OLOFP method for the correct parameter case and performed better for the plant/model mismatch case. In this section, we will explain in more detail the effect of cost factor on the process operation and optimality.

Since the CLOC performed equally or even better than OLOFP method, the control simulation in this section will be performed using the CLOC method. For convenient on discussion, Equation (5-14), which describes the optimal substrate profile and Equation (5-15), which describes the relationship between biomass and substrate concentration during the optimal period are written here:

$$\dot{S} = -\frac{\mu'(\pi'\mu - \pi\mu')}{(\mu'\pi'' - \pi'\mu'')} \qquad (5\text{-}14)$$

$$X = -\frac{\mu'\varepsilon}{(\pi'\mu - \pi\mu')} \qquad (5\text{-}15)$$

As mentioned earlier, the optimal substrate concentration profile in Equation (5-14) is only a function of the substrate concentration. The process invokes into the optimal period and following the optimal trajectory by the condition in Equation (5-15). The profile of optimal substrate concentration which is shown in Figure 5-13 shows that the optimal profile is bounded by two levels of substrate concentration. These bounds can be determined by Equation (5-15) following two conditions below:

$$\mu' = 0 \tag{5-17}$$

or

$$\pi'\mu - \pi\mu' = 0$$

which implies that,

$$\frac{d(\pi/\mu)}{dS} = 0 \tag{5-18}$$

The first condition (Equation (5-17)) means that the substrate concentration is kept at a level, which maximises the biomass growth rate. This results in the shortest process operating time. The second condition (Equation (5-18)) means that the substrate concentration is kept at a level, which maximises the ratio between the specific product formation rate (π) and the specific growth rate (μ). This condition is similar to Equation (3-63) in Chapter 3 and Equation (4-31) in Chapter 4 in which there is no cost factor in the objective function. Hence it results in the longest operating time.

These constant substrate concentration levels are upper and lower bounds for the optimal substrate concentration in Equation (5-14) and Figure 5-13. These two boundaries also represent two extreme conditions. The first is where cost factor is not important at all and we are interested only on maximising the ratio of π and μ. This results in maintaining

substrate concentration under the condition in Equation (5-18). The other is where the cost factor is very important and we want the shortest operating time. This results in maintaining substrate concentration under the condition in Equation (5-17), which maximises the biomass growth rate.

To demonstrate the effect of cost factor on the process operating, a set of simulations at different cost factors are performed. These cost factors are 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and maximum. The maximum here means that the cost factor is very important and the process operating time needs to be as short as possible. Therefore in this case, the substrate concentration will be kept constant at the point which maximises the biomass growth rate (5 g/l). The relationship between the specific growth rate, the specific product formation rate and the ratio between both rates (π/μ) are shown in Figure 5-23. The simulation results on the various cost factors are shown in Figure 5-24 to Figure 5-30.

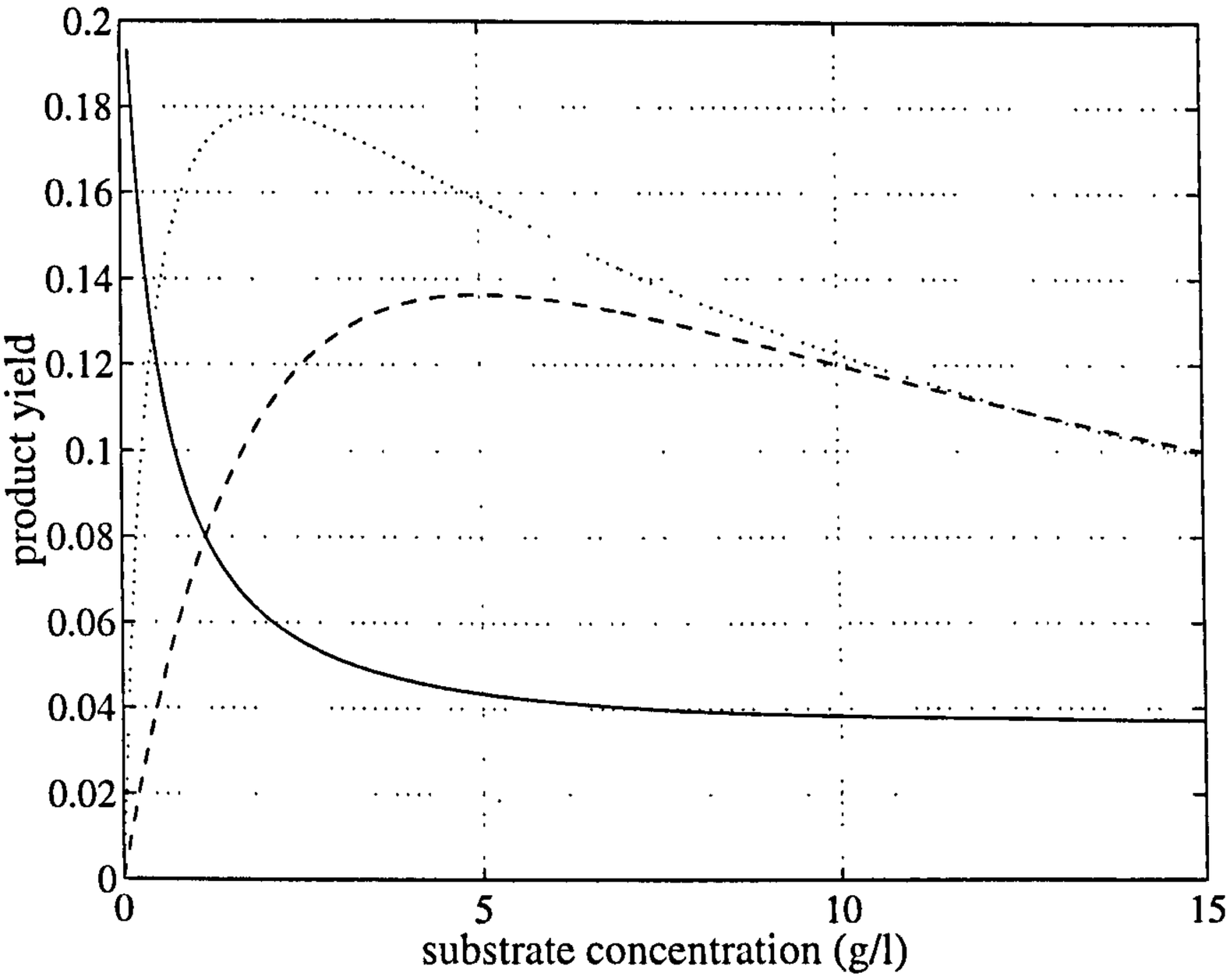
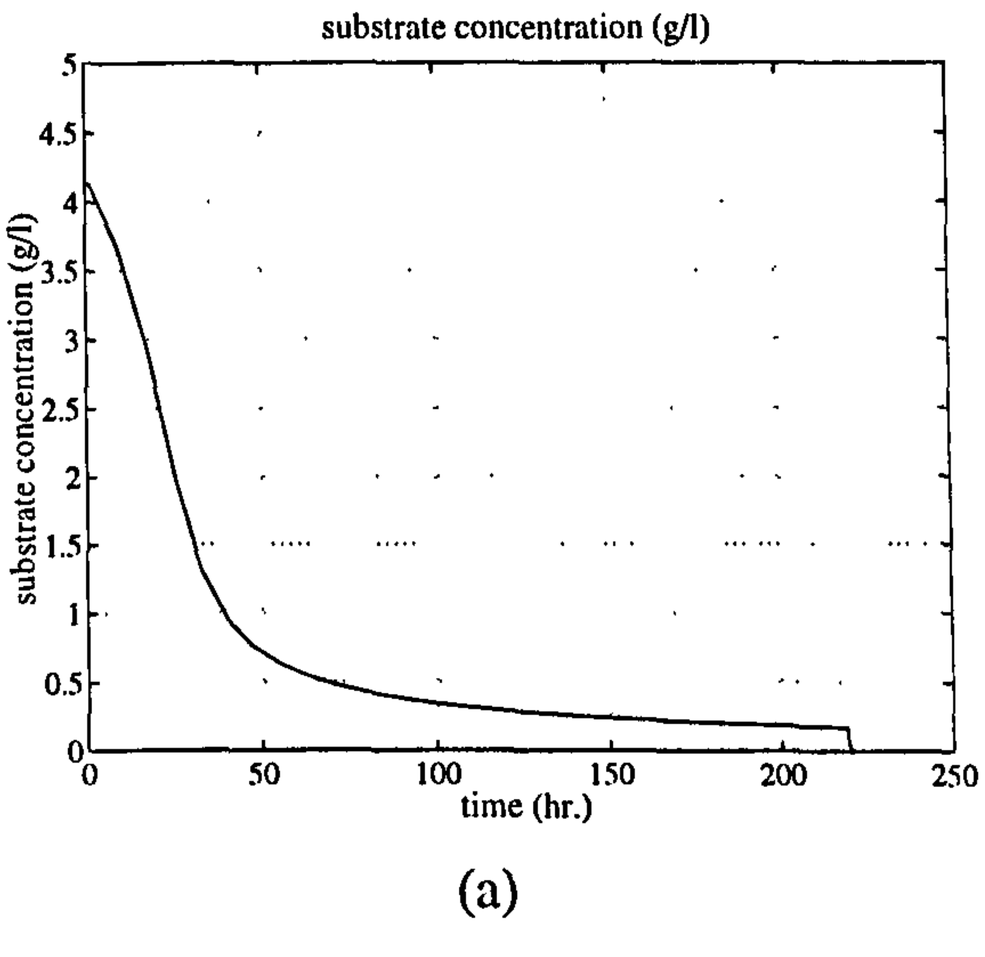


Figure 5-23 Relationship between product yield and substrate concentration

The specific growth rate is shown with dashed line while the specific product formation rate is shown with dotted line in the figure. The ratio between both rates (π/μ) is shown with solid line. Note that these rates are scaled to fit into the figure.



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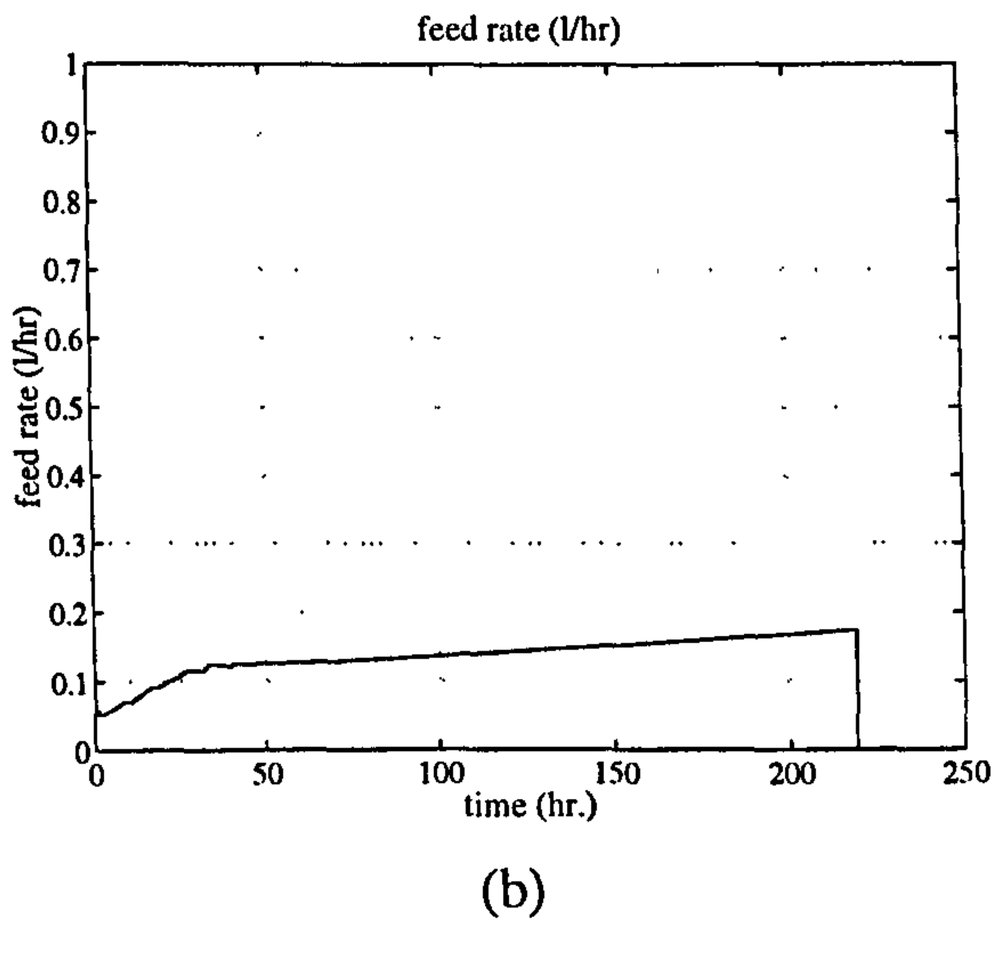

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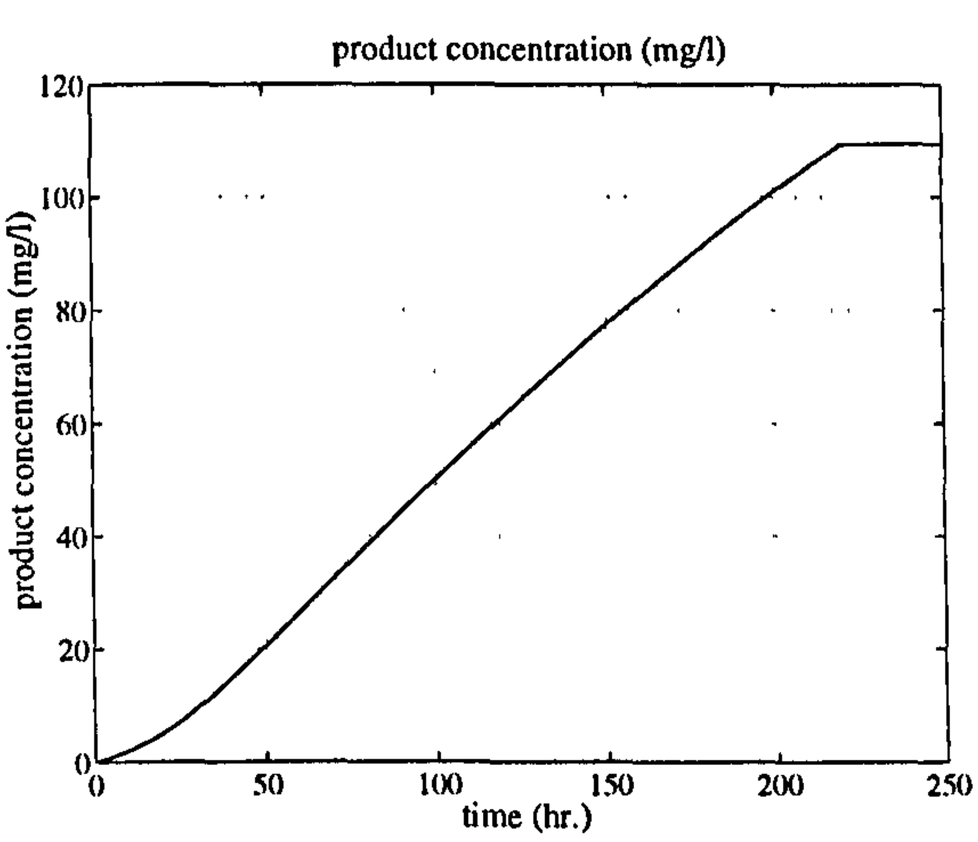

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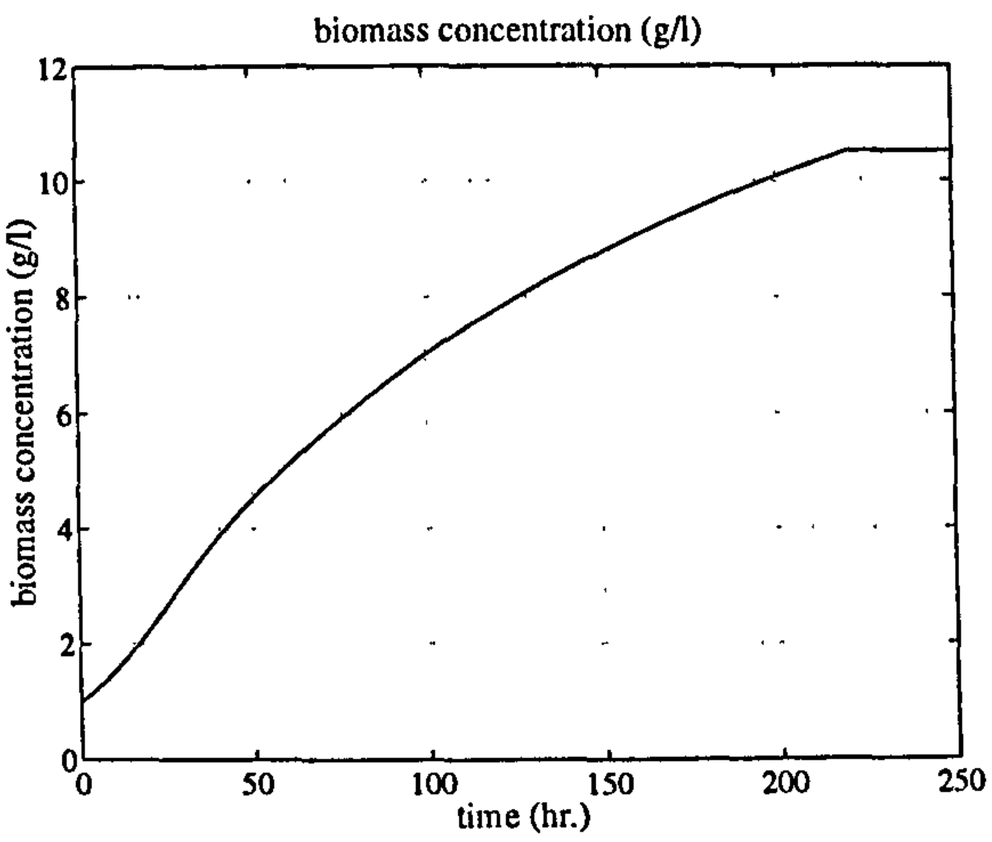

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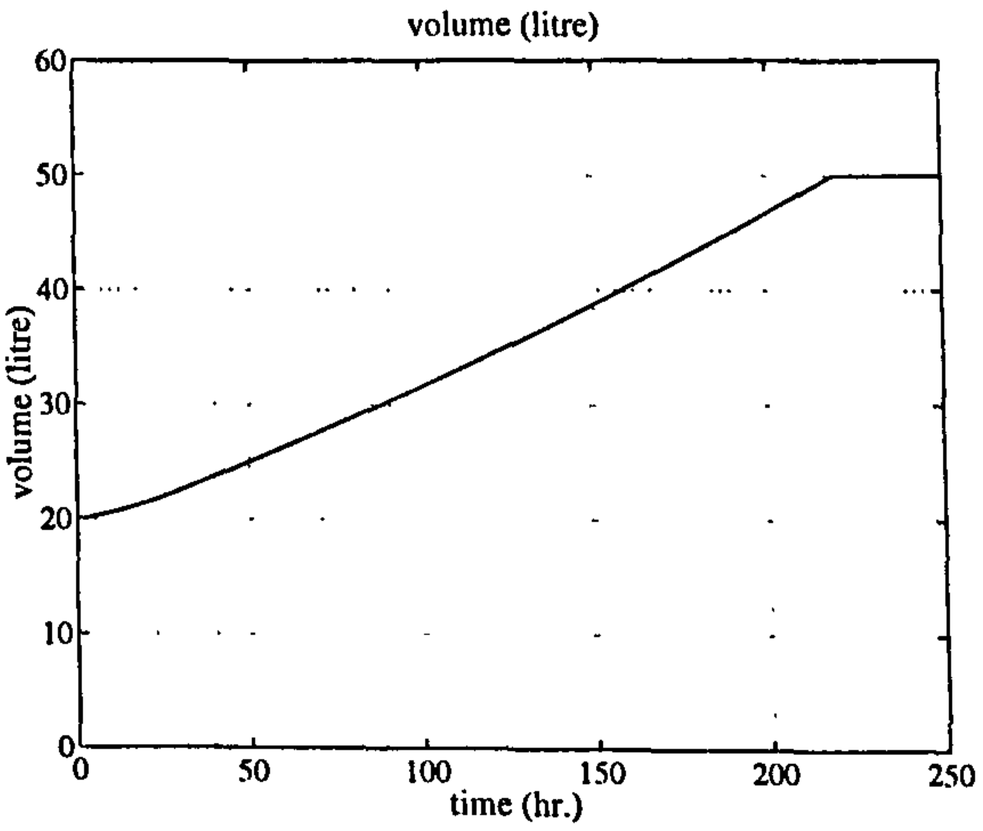
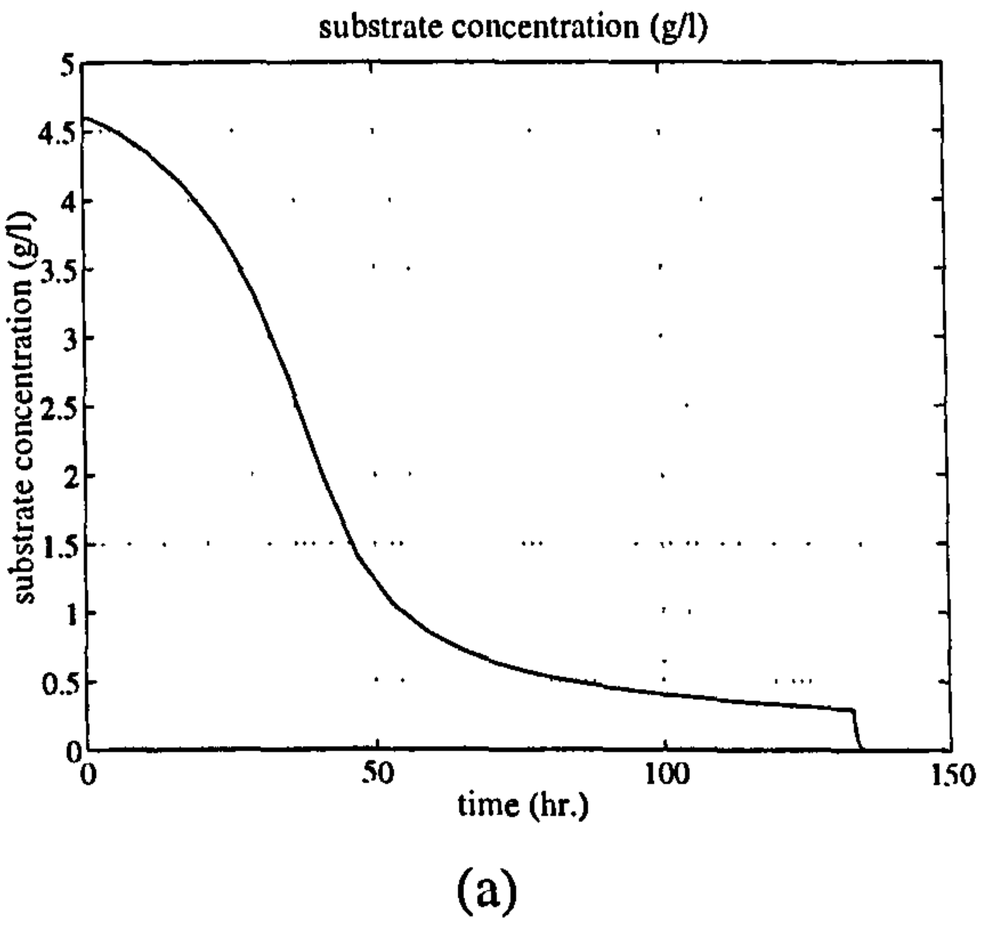


(e)

Figure 5-24 Simulation results of a secondary metabolite production using cost factor equals 0.5



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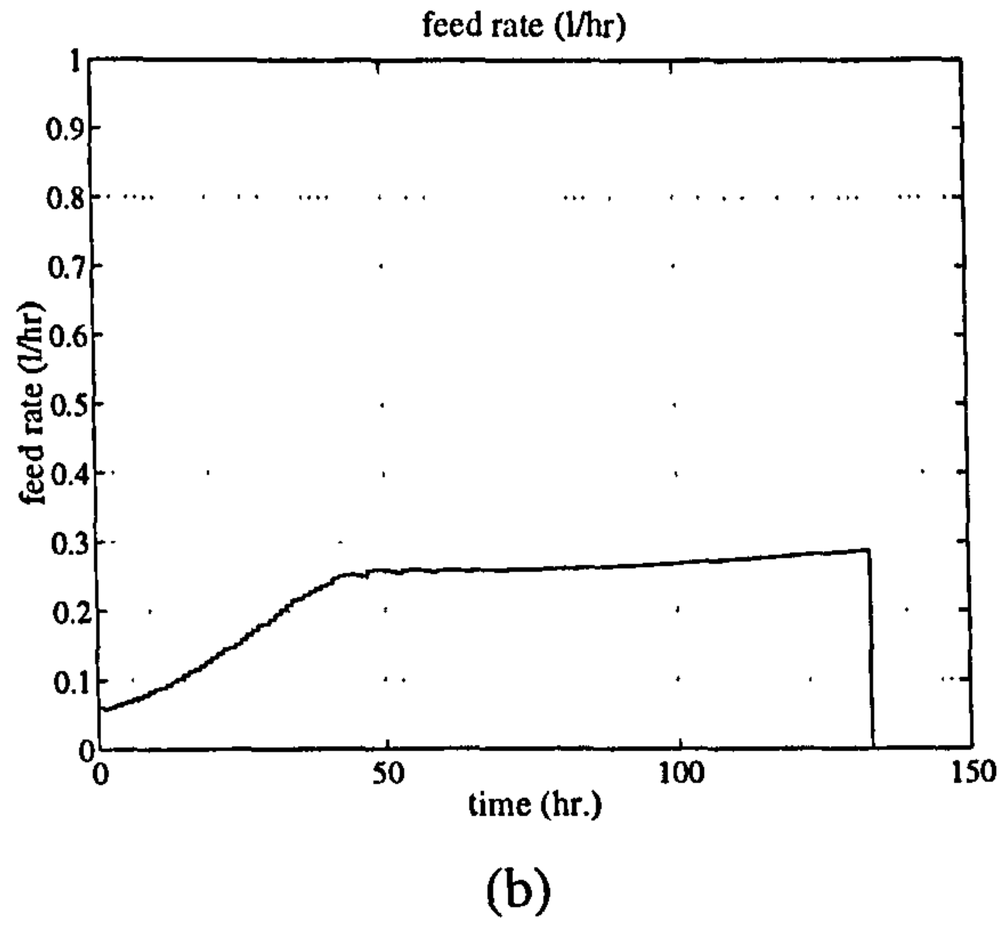

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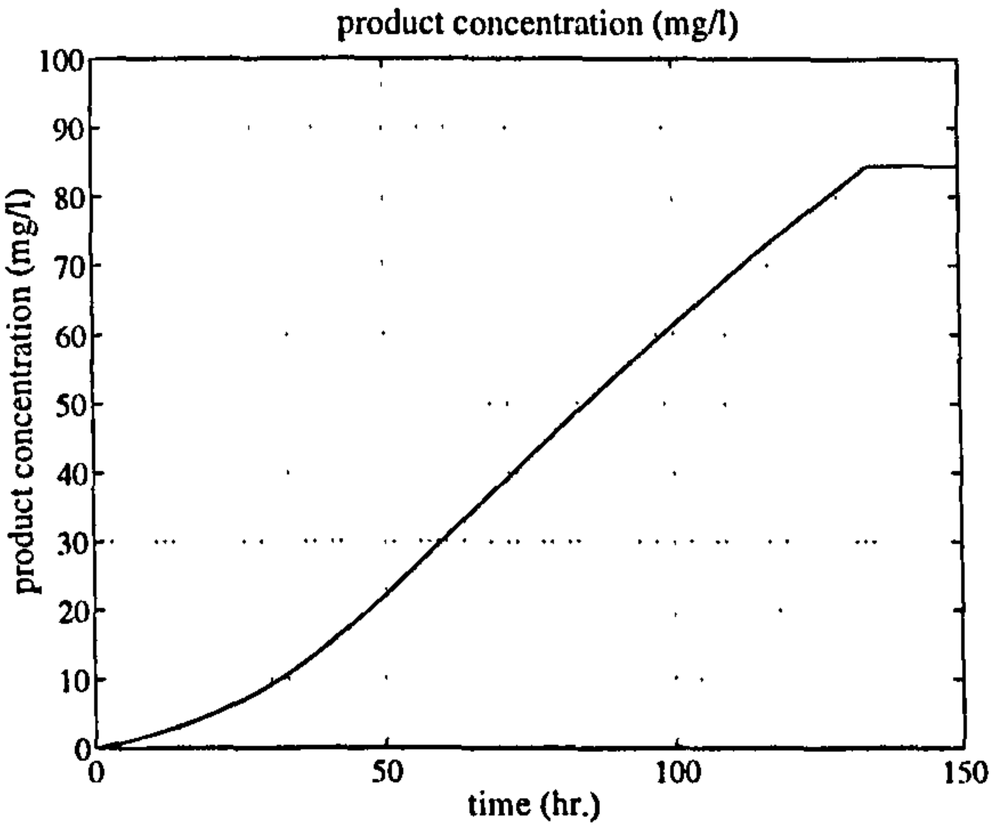

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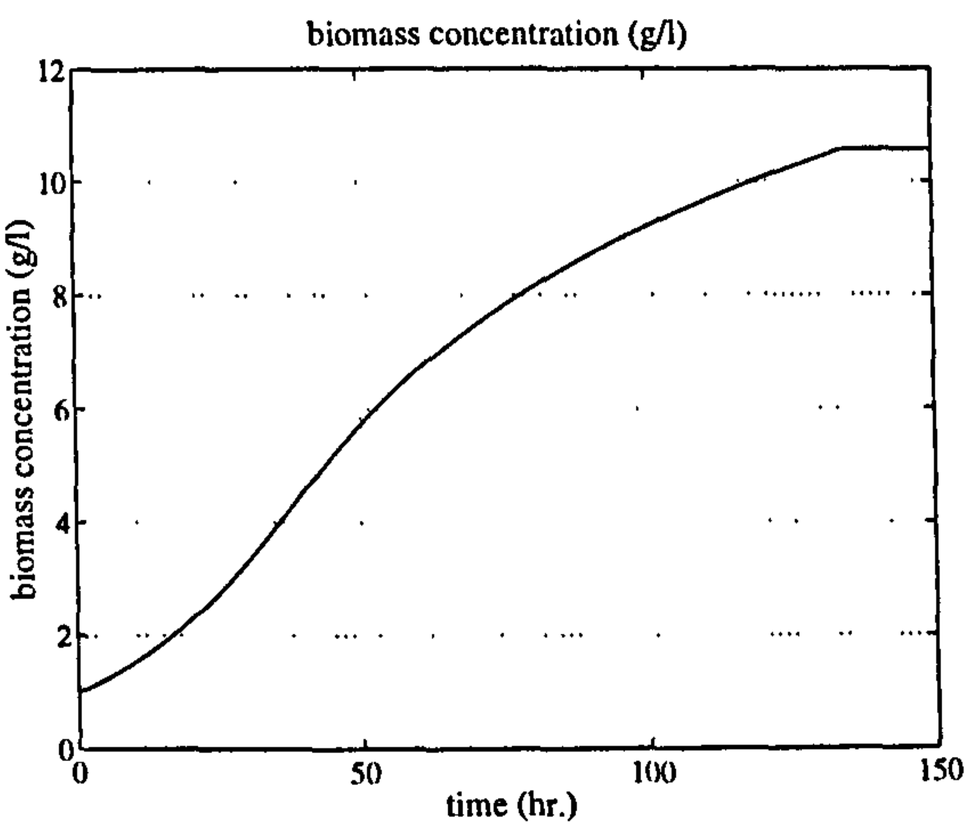

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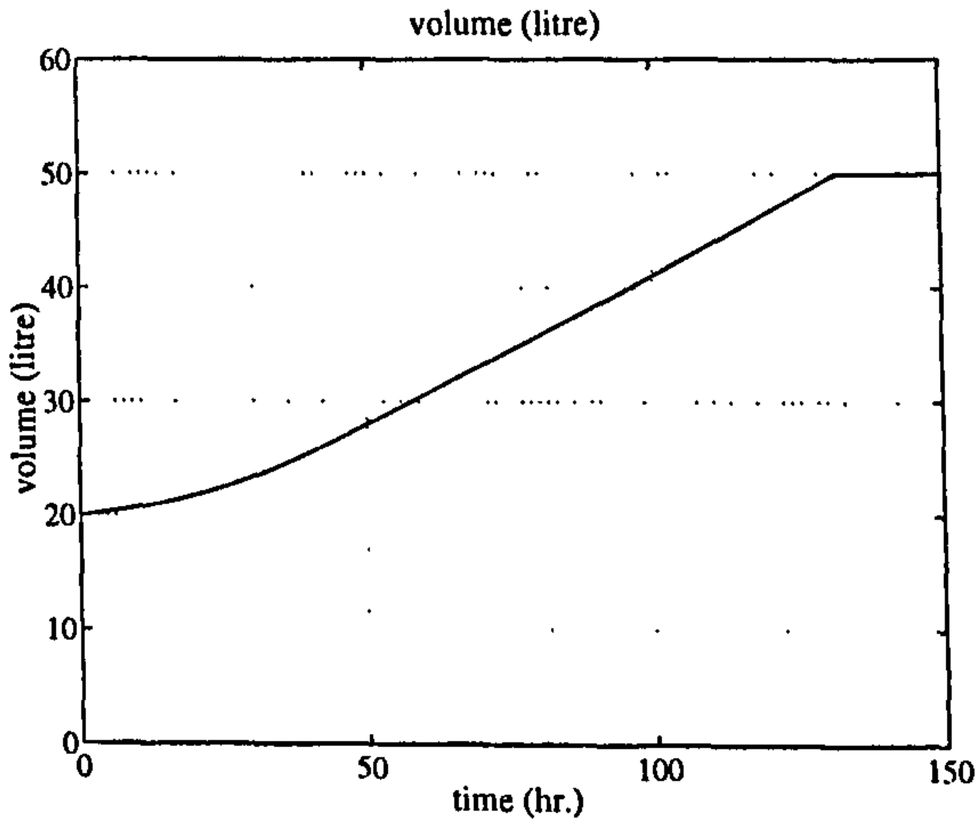
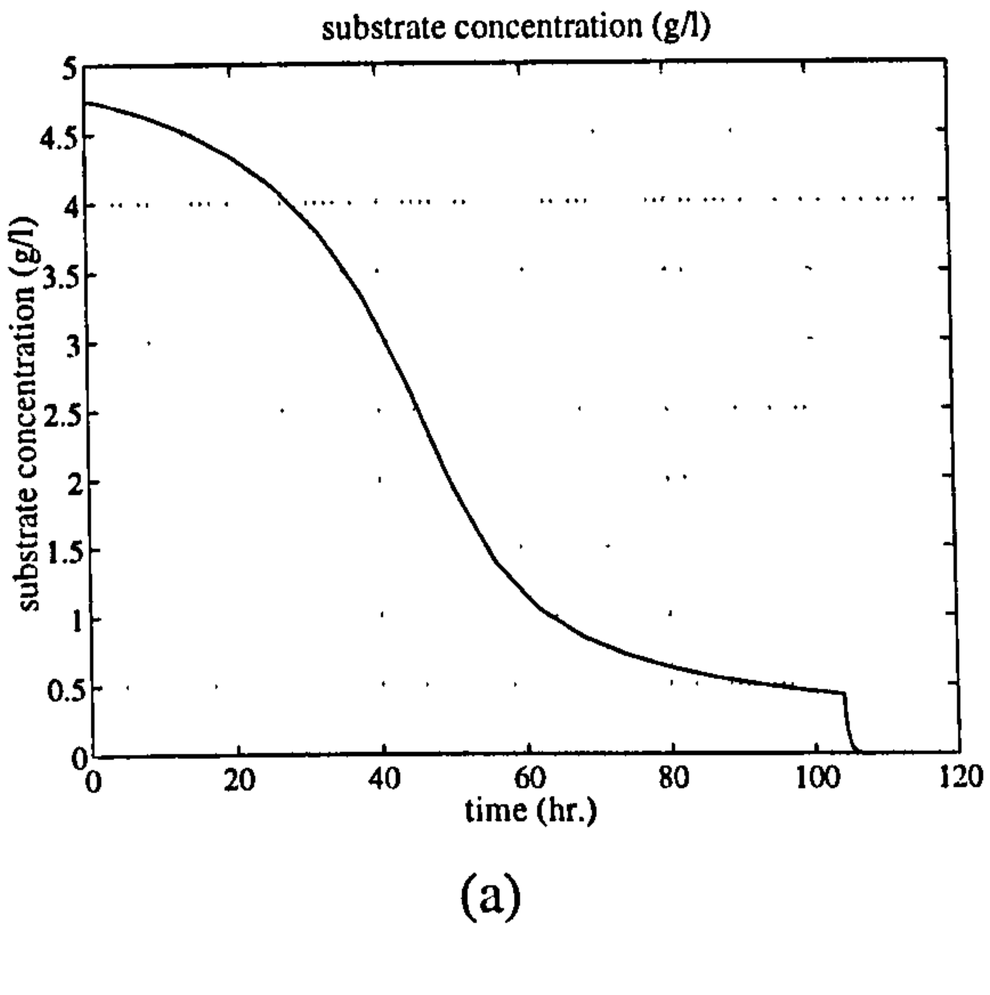


(e)

Figure 5-25 Simulation results of a secondary metabolite production using cost factor equals 1.0



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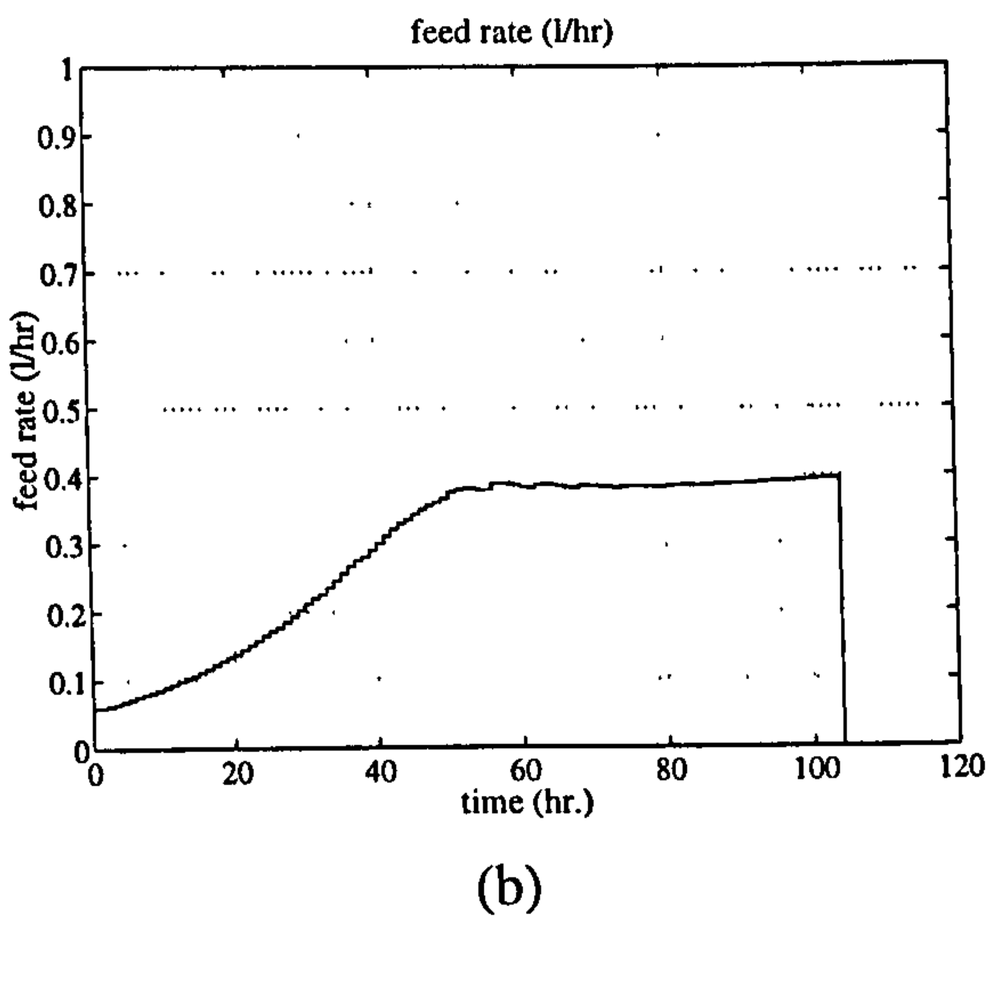

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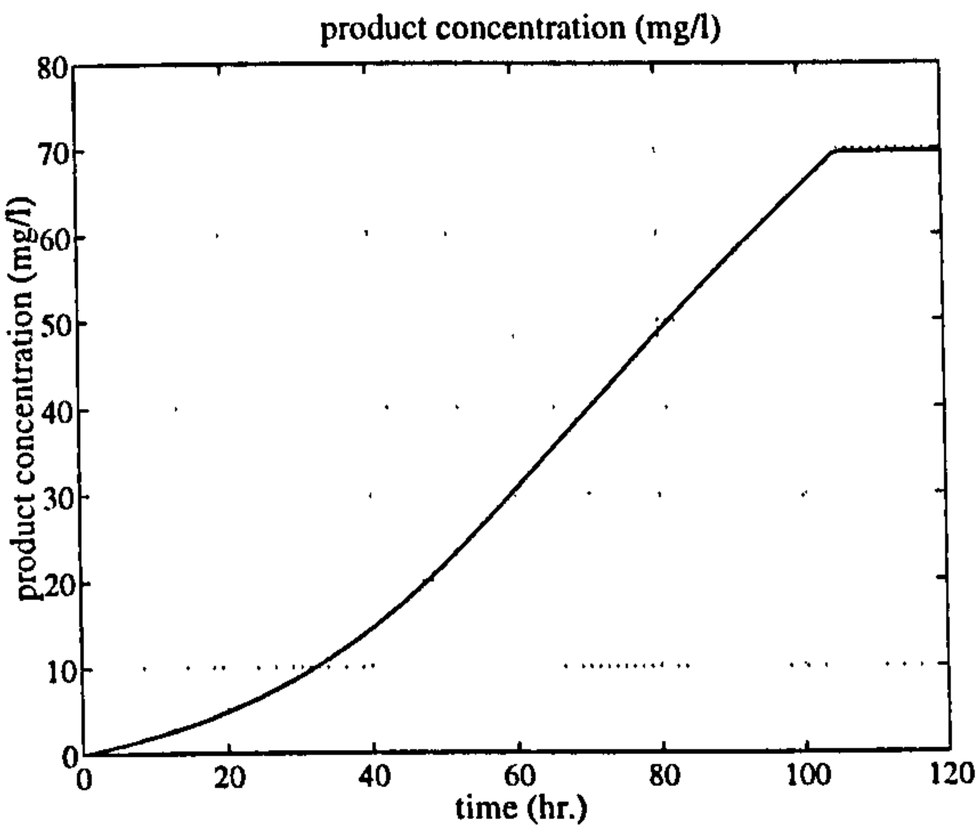

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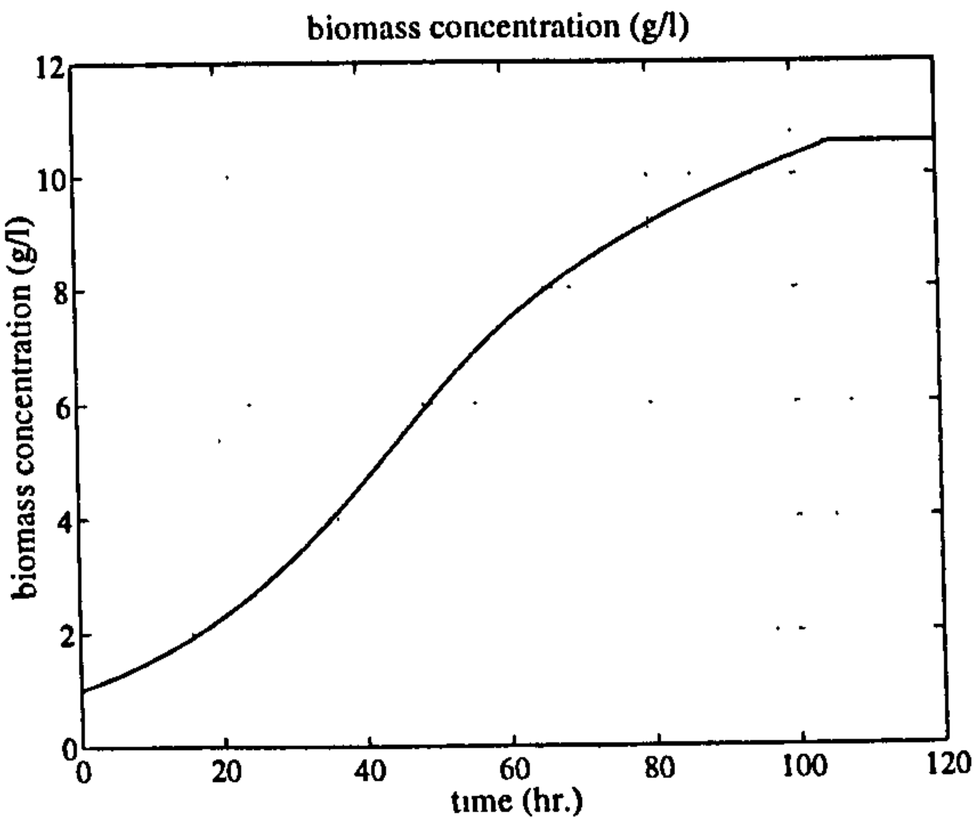

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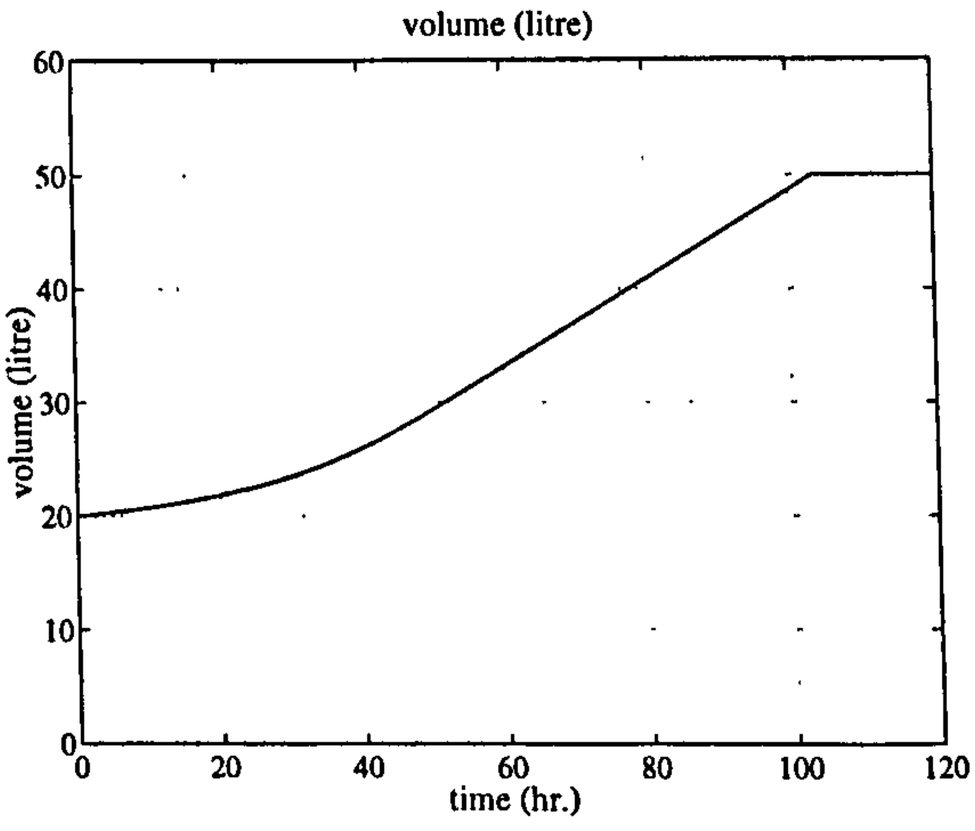


(e)

Figure 5-26 Simulation results of a secondary metabolite production using cost factor equals 1.5

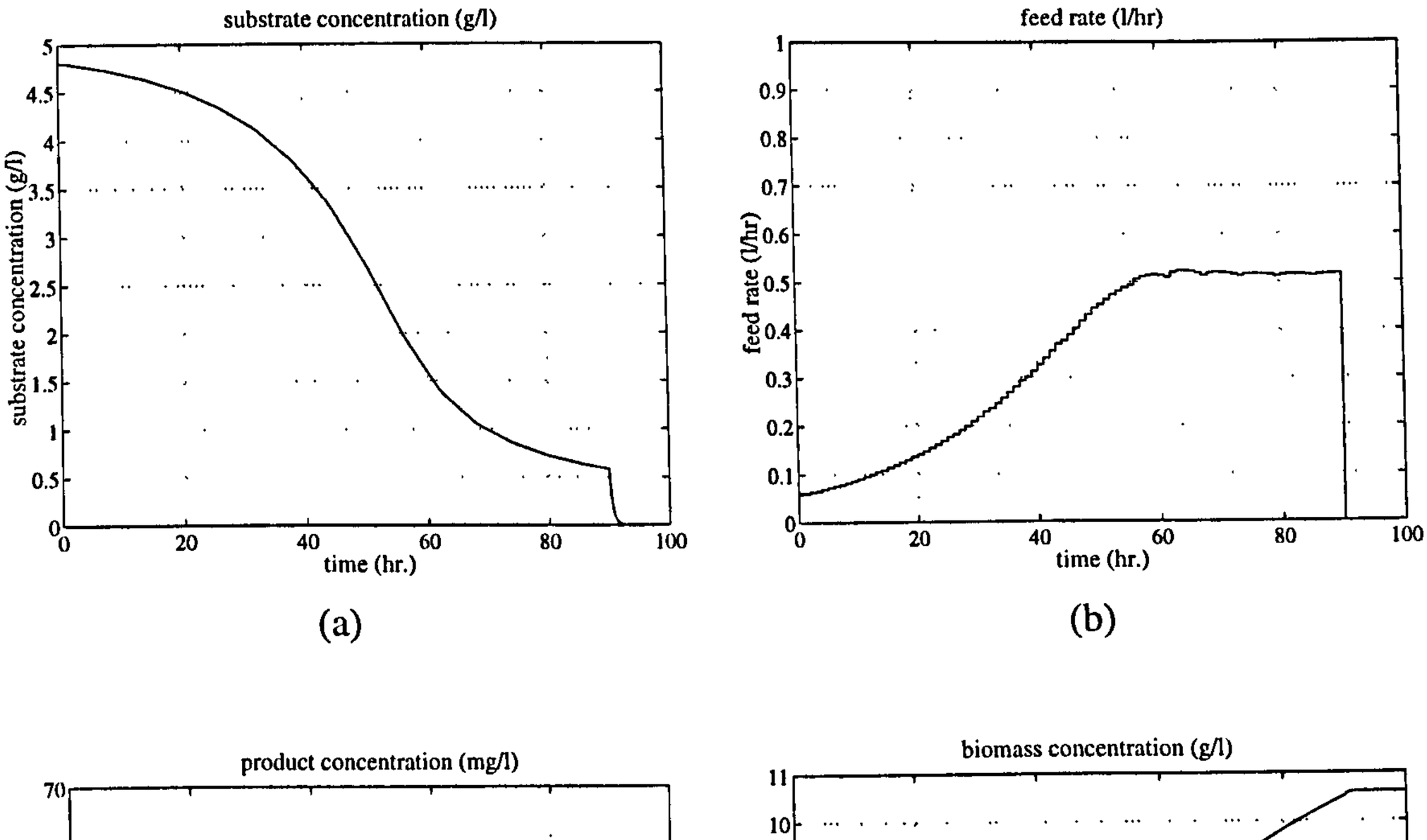
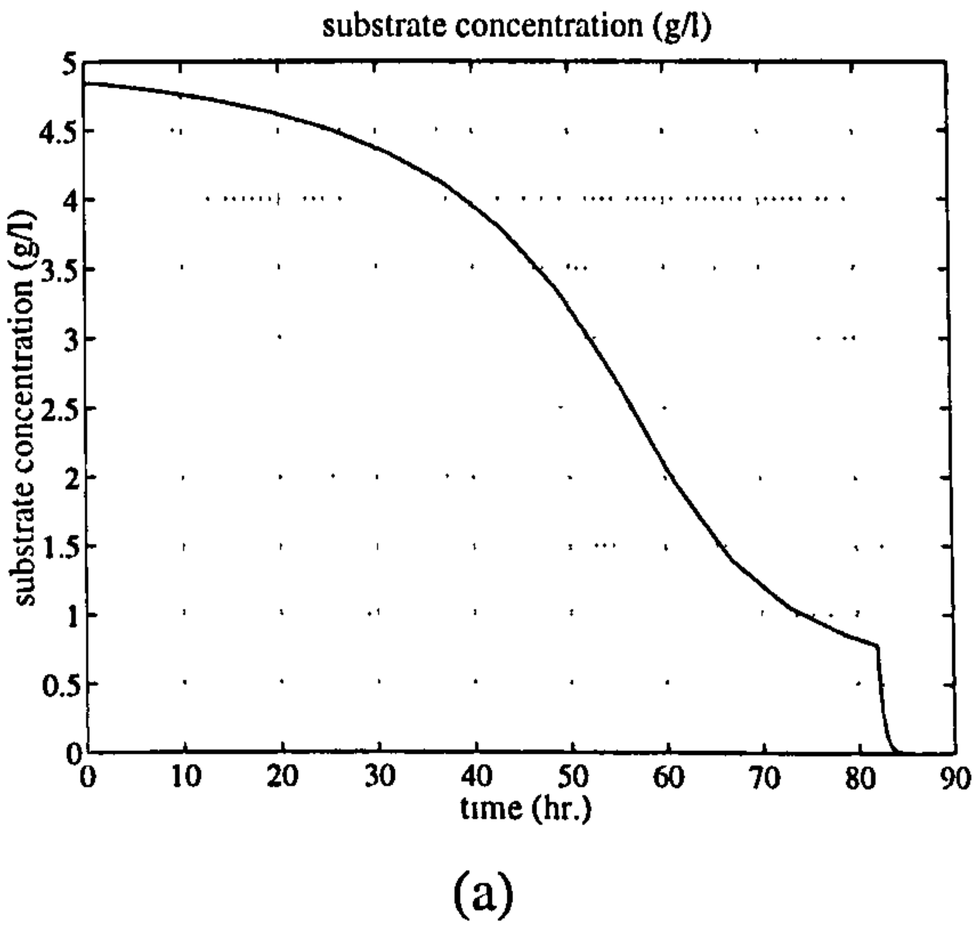


Figure 5-27 Simulation results of a secondary metabolite production using cost factor equals 2.0



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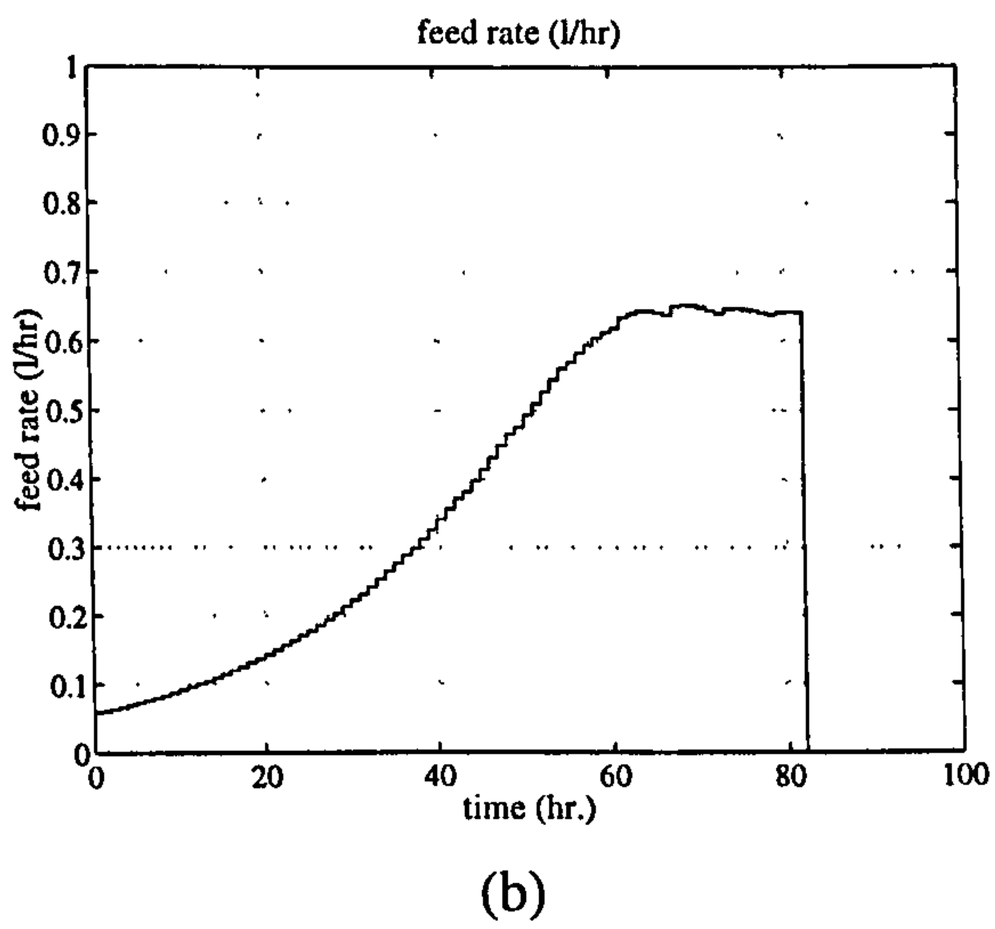

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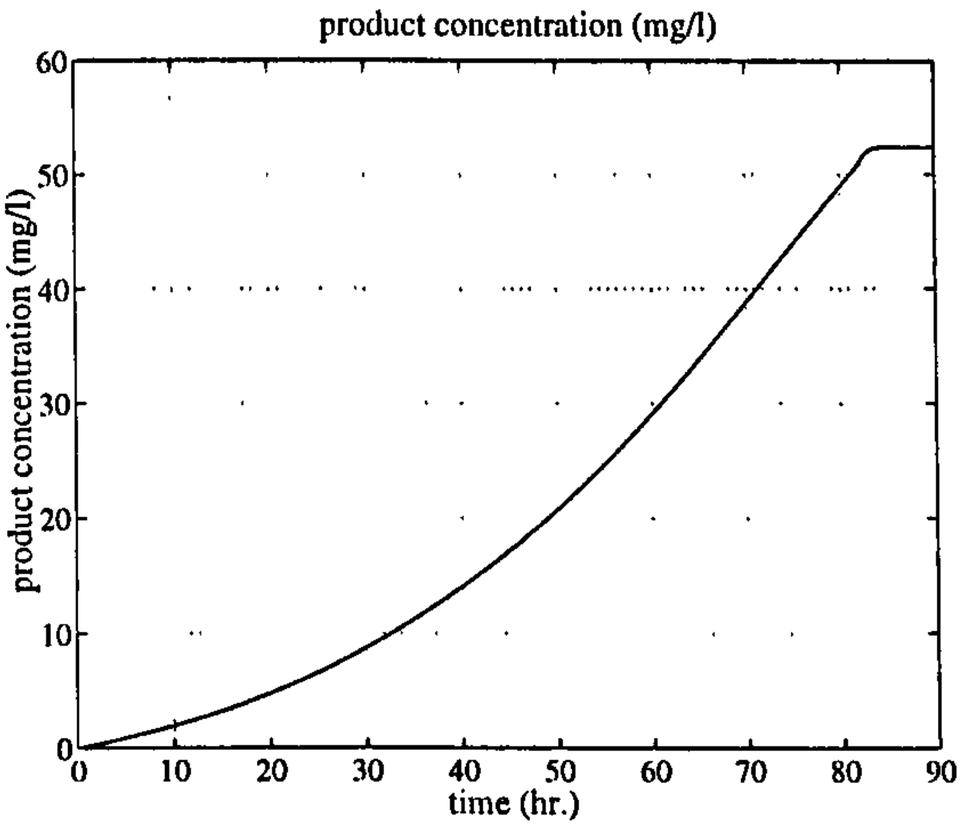

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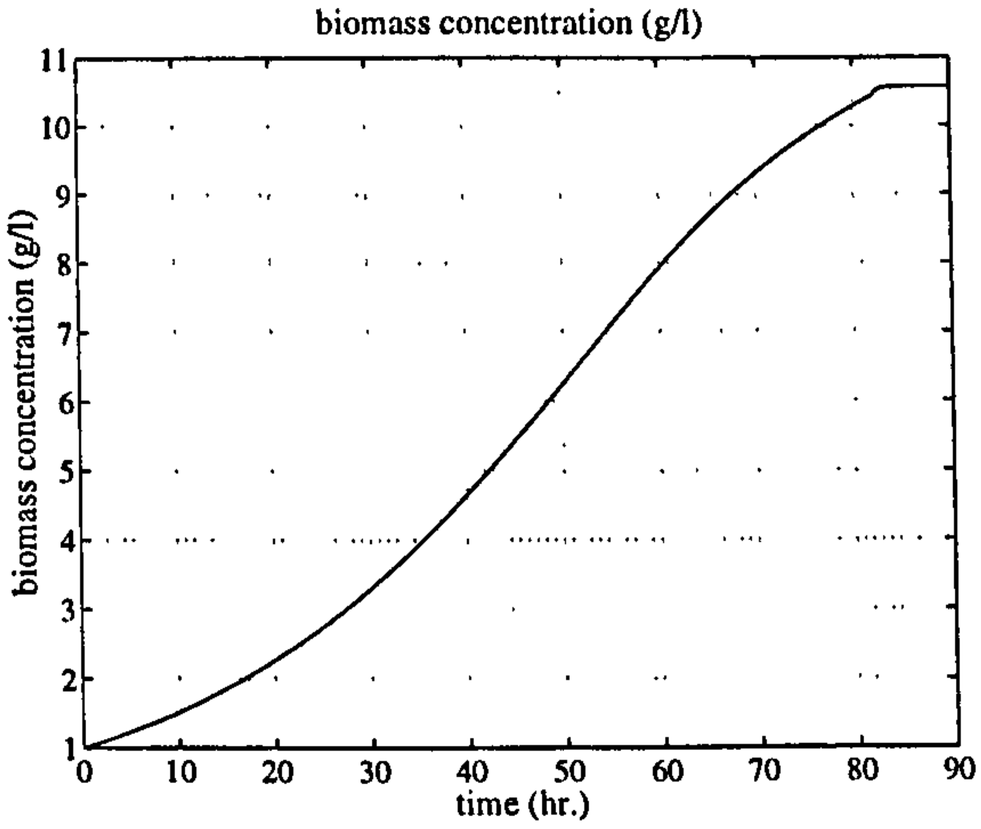

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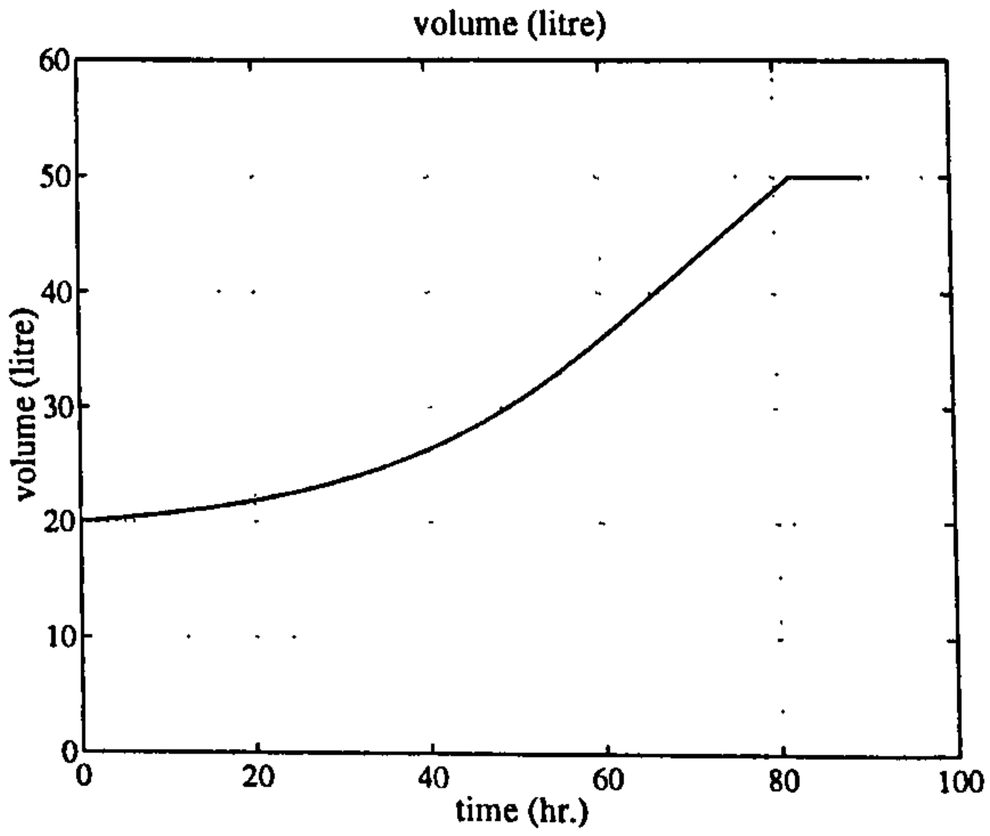
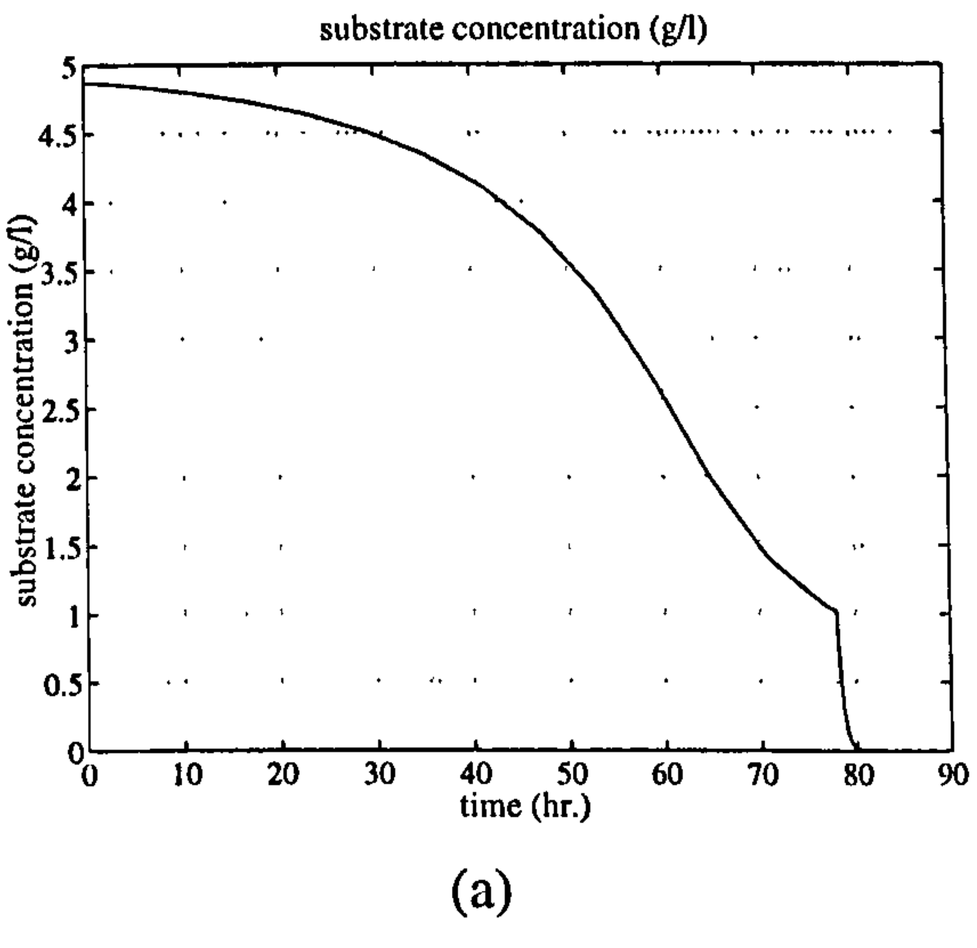
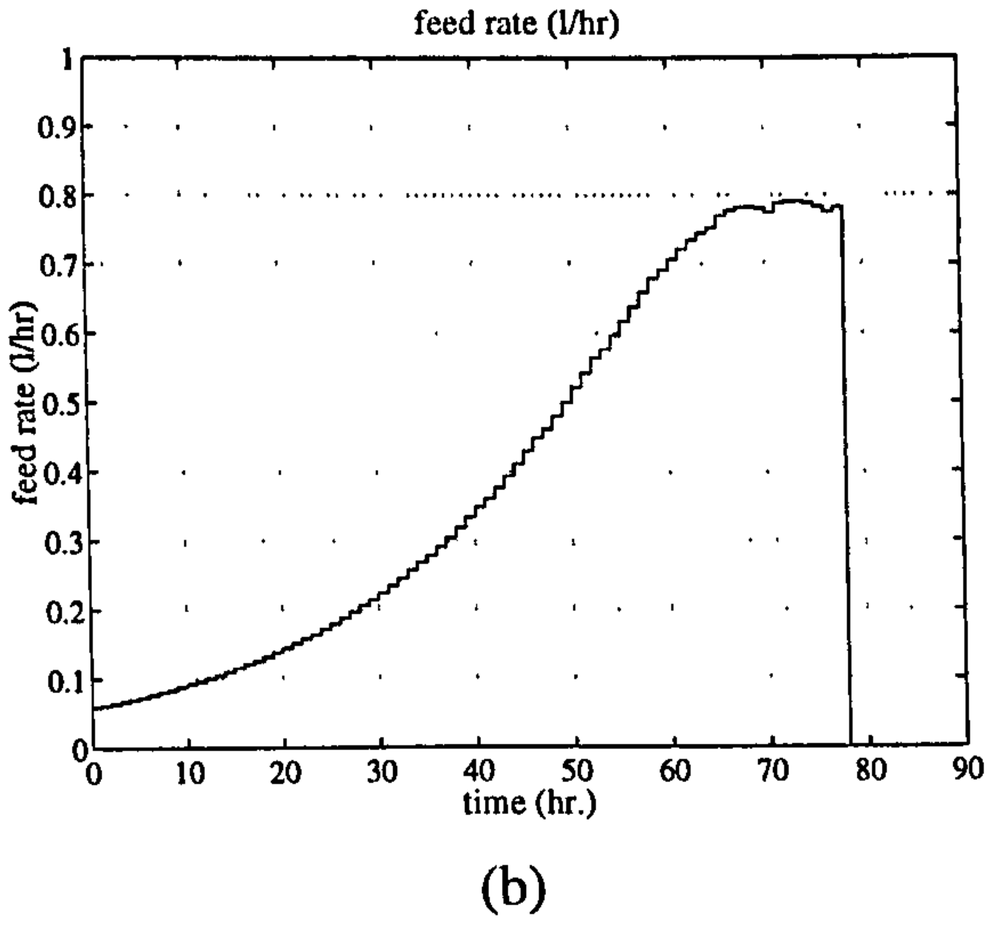


(e)

Figure 5-28 Simulation results of a secondary metabolite production using cost factor equals 2.5



(a)



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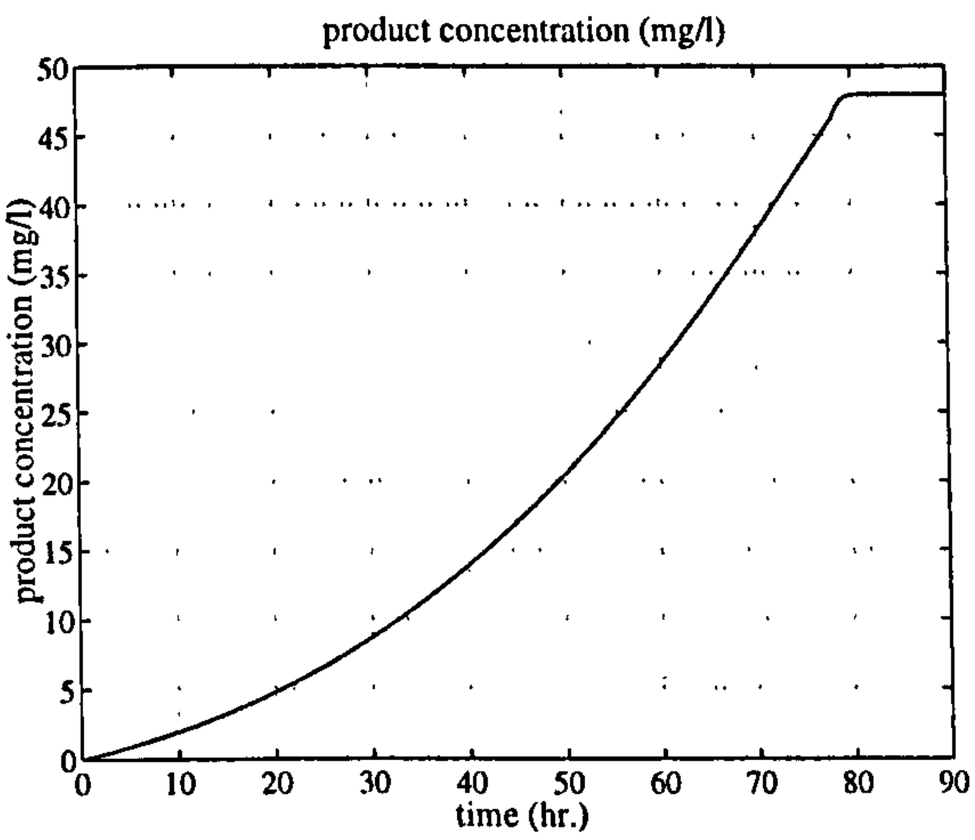

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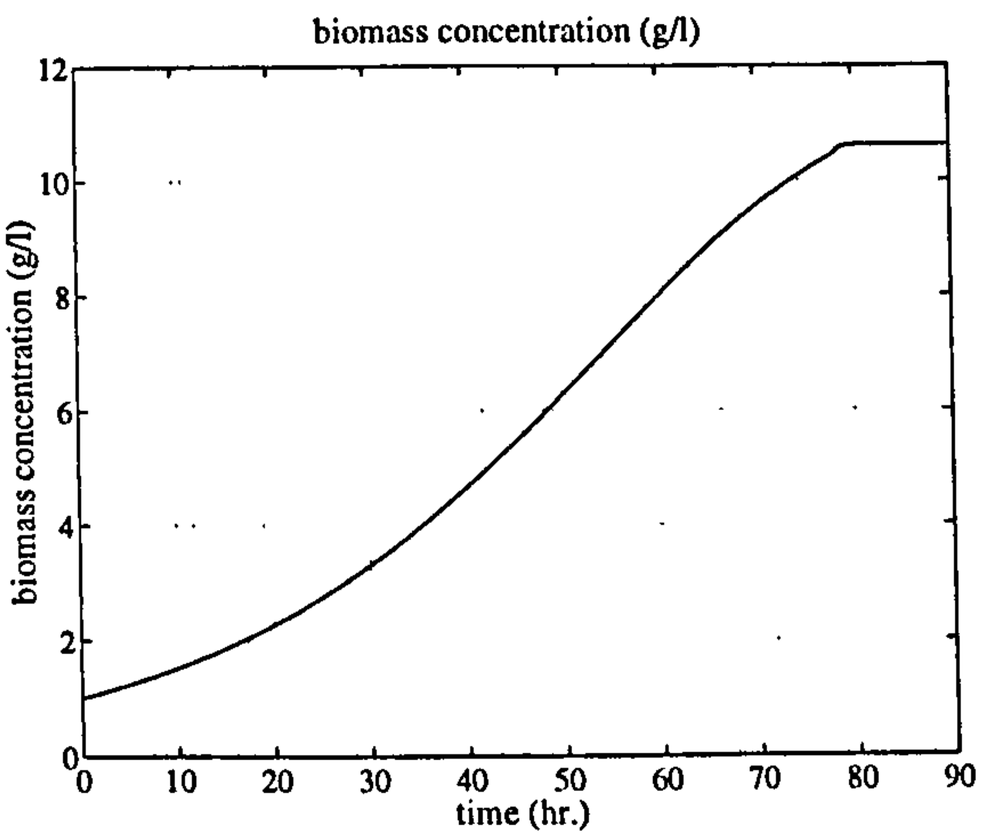

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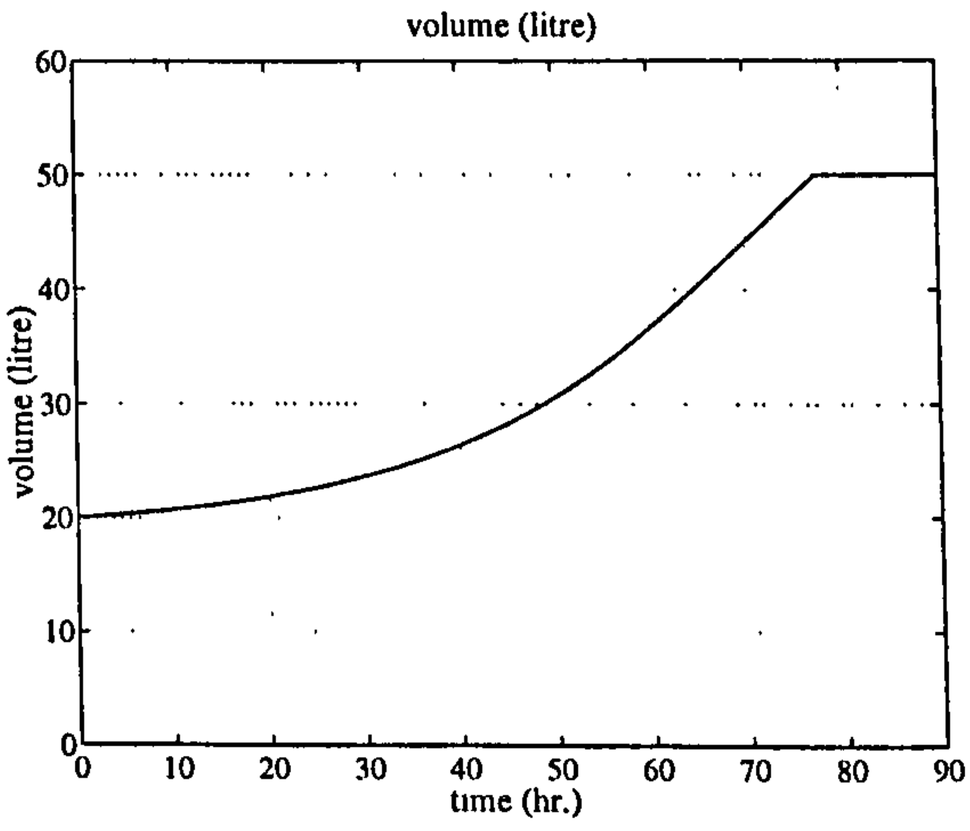
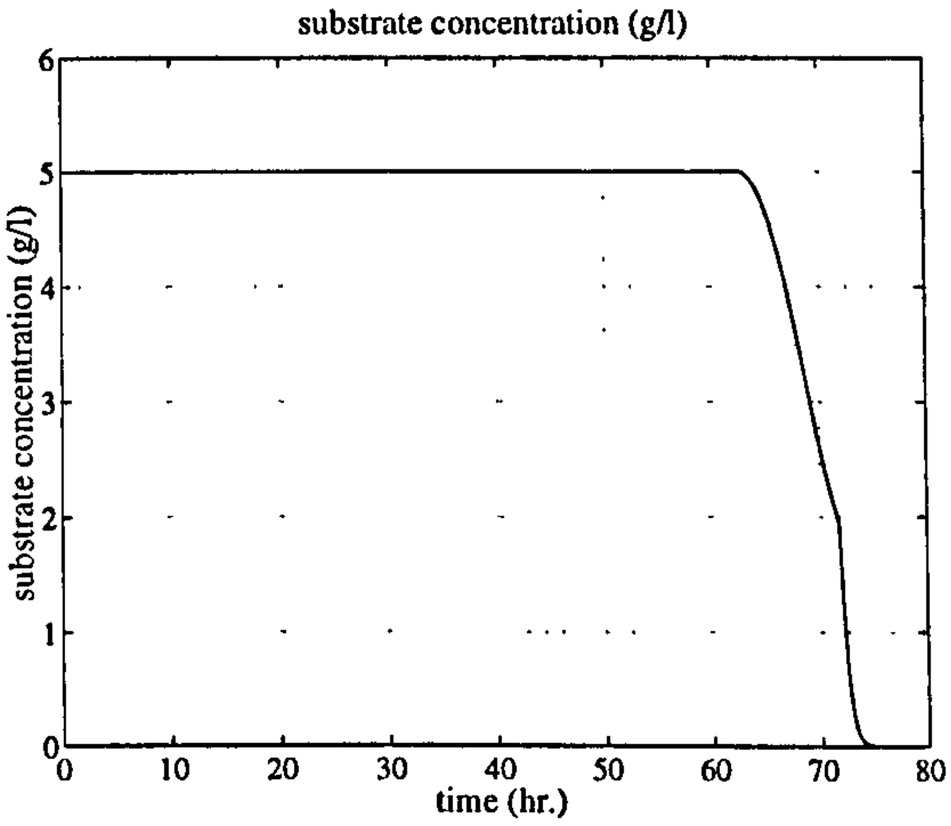
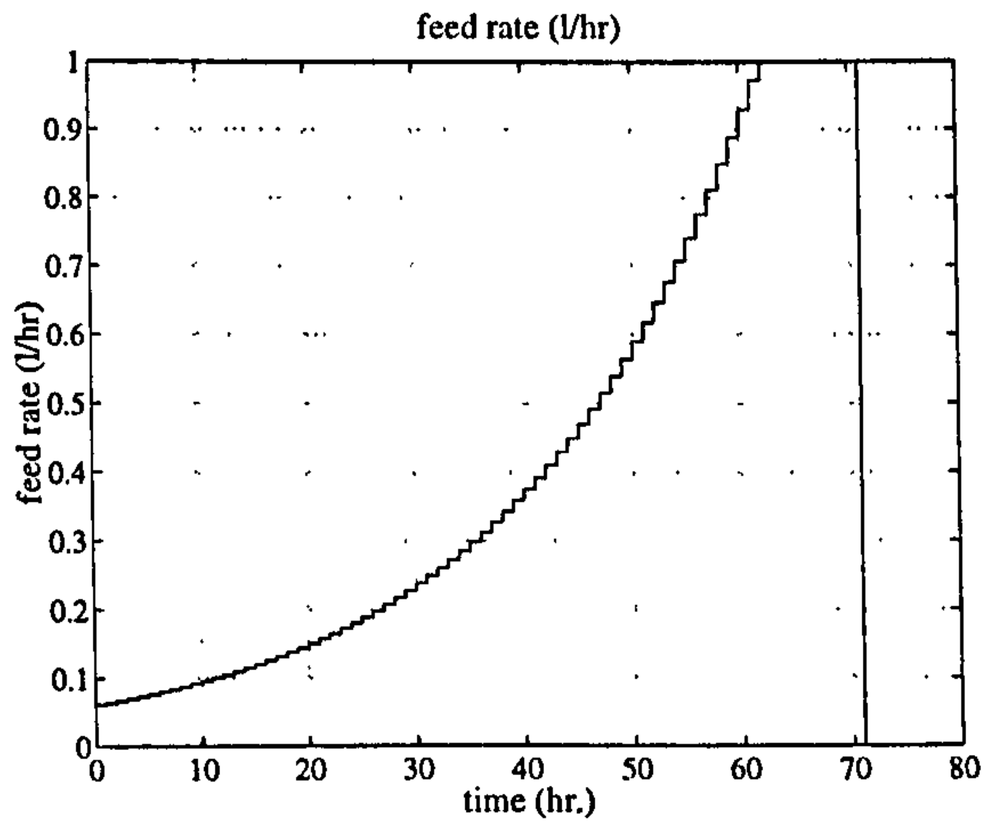


(e)

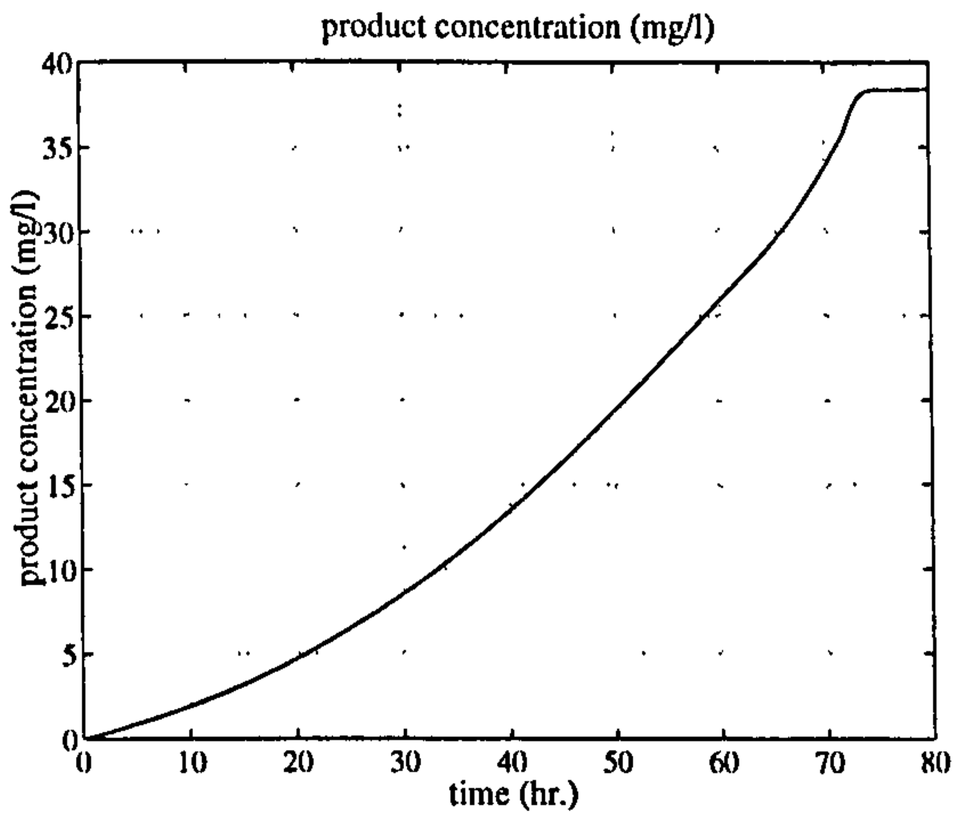
Figure 5-29 Simulation results of a secondary metabolite production using cost factor equals 3.0



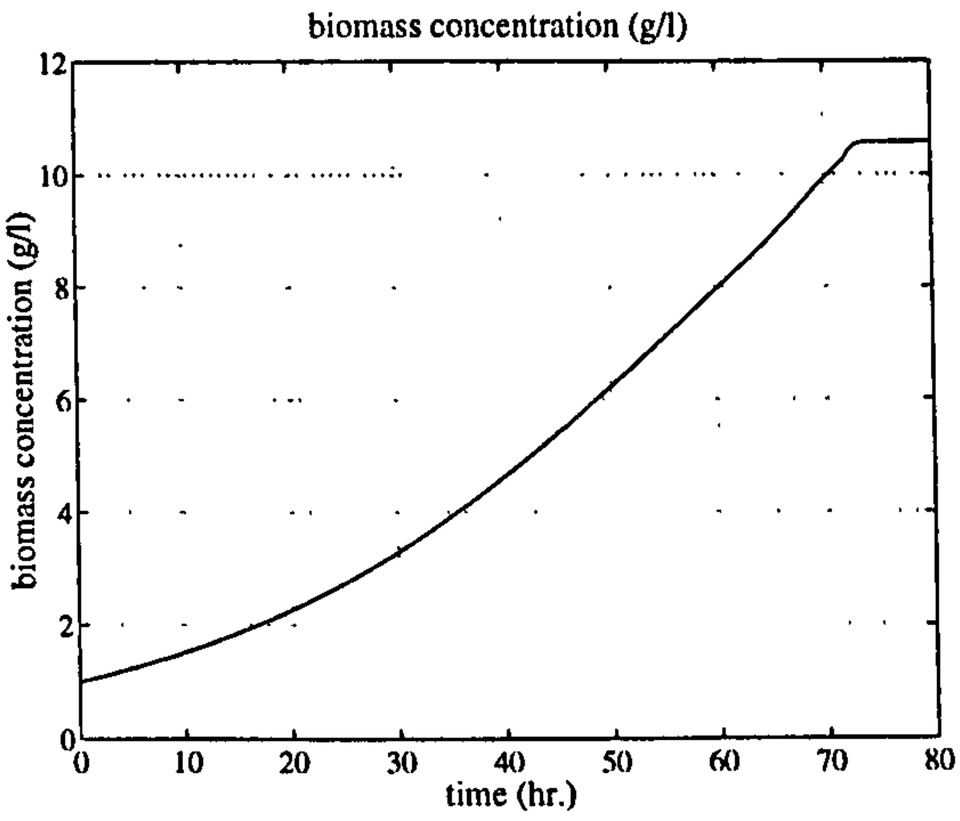
(a)



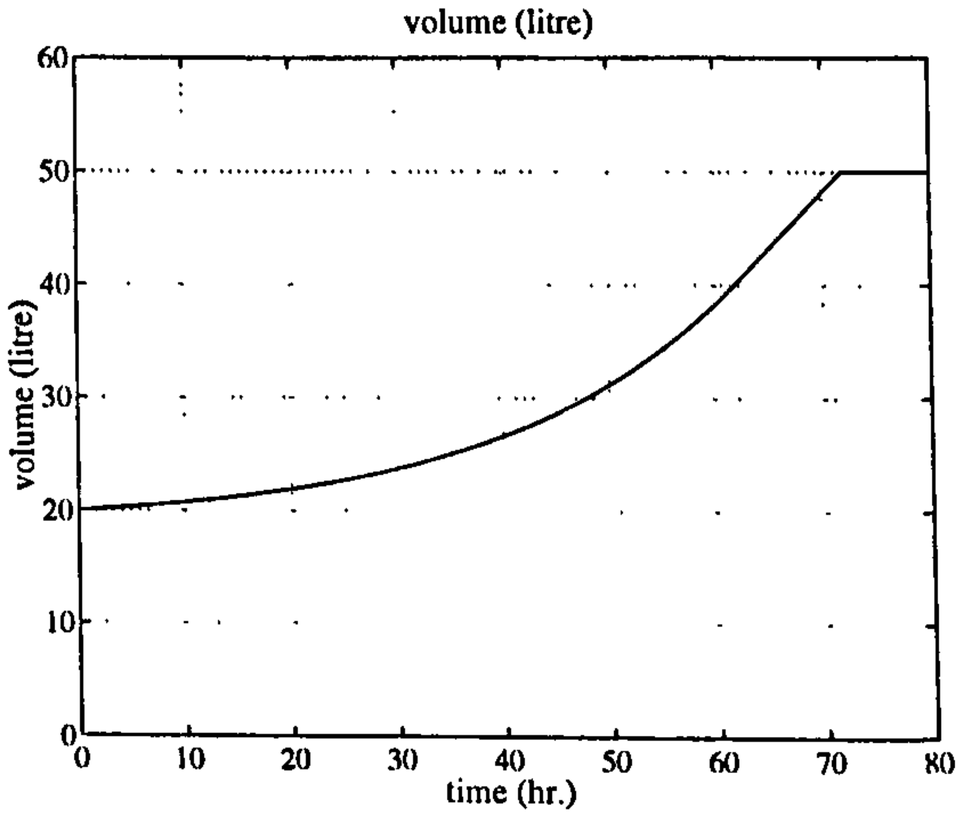
(b)



(c)



(d)



(e)

Figure 5-30 Simulation results of a secondary metabolite production using cost factor at maximum

As the cost factor increases, the operating time becomes shorter to satisfy the objective function. This can also be seen from the increasing of feed rate during the batch and the faster growing of biomass. The operating time, maximum product, maximum biomass and yields at different cost factors are shown in Table 5-8.

Table 5-8 Summary of simulation results at different cost factor

Cost factor	0.5	1.0	1.5	2.0	2.5	3.0	max.
Substrate (g/l)	4.132	4.596	4.735	4.80	4.84	4.87	5.0
Product (mg/l)	109.34	84.42	69.63	59.70	52.40	47.95	38.39
Biomass (g/l)	10.51	10.57	10.57	10.61	10.57	10.61	10.56
Time (hr.)	219	134	104	90	82	78	71
Y_{px} (mg product/g biomass)	10.40	7.99	6.59	5.63	4.96	4.52	3.64
Y_{sx} (g substrate/g biomass)	5.87	5.85	5.86	5.84	5.86	5.84	5.87
Y_{ps} (mg product/g substrate)	1.77	1.37	1.12	0.96	0.85	0.77	0.62

In the table, substrate means the initial substrate concentration at beginning of the batch. The difference in the initial substrate concentration levels is due to the fact that with the different cost factor, the optimal relationship between biomass and substrate concentration is different as shown in Figure 5-14. However, the initial biomass concentration used for all simulations at different cost factors is 1 g/l. The initial substrate concentration is therefore chosen to correspond to the initial biomass concentration (1 g/l) at different cost

factors. Product is the maximum product obtained at the end of the process. Time is the process operating time. Biomass is biomass concentration at the end of the process.

Y_{ps} is an average yield of product from biomass. It shows the amount of product obtained per unit of biomass and can be calculated by the following equation:

$$Y_{px} = \frac{P(t_f) \cdot V(t_f)}{X(t_f) \cdot V(t_f)} \qquad (5\text{-}19)$$

Y_{sx} is an average yield of biomass from substrate. It shows the amount of substrate that is used by a unit of biomass and can be determined from the following equation:

$$Y_{sx} = \frac{S(t_i) \cdot V(t_i) + \left(V(t_f) - V(t_i)\right) S_f}{X(t_f) \cdot V(t_f)} \qquad (5\text{-}20a)$$

This equation is used to calculate Y_{sx} in Table 5-8.

Y_{sx} is also referred to as the amount of substrate concentration that is used to produce a unit of biomass. In this meaning, it is determined by the following equation:

$$Y_{sx} = \frac{S(t_i) \cdot V(t_i) + \left(V(t_f) - V(t_i)\right) S_f}{X(t_f) \cdot V(t_f) - X(t_i) \cdot V(t_i)} \qquad (5\text{-}20b)$$

Note that Y_{sx} in Equation (5-20b) is a conversion of Y_{xs} used in the simulation.

Y_{ps} is an average yield of product from substrate. It shows the amount of product that can be obtained from using a unit of substrate and can be determined from the following equation, which is the proportion of Y_{px} and Y_{sx} ;

$$Y_{ps} = \frac{Y_{px}}{Y_{sx}} \qquad (5\text{-}21)$$

To show the effect of cost factor on the process, the maximum product and yields (Y_{px}, Y_{sx} and Y_{ps}) are plotted against the operating time as shown in Figure 5-31 and Figure 5-33 to Figure 5-35.

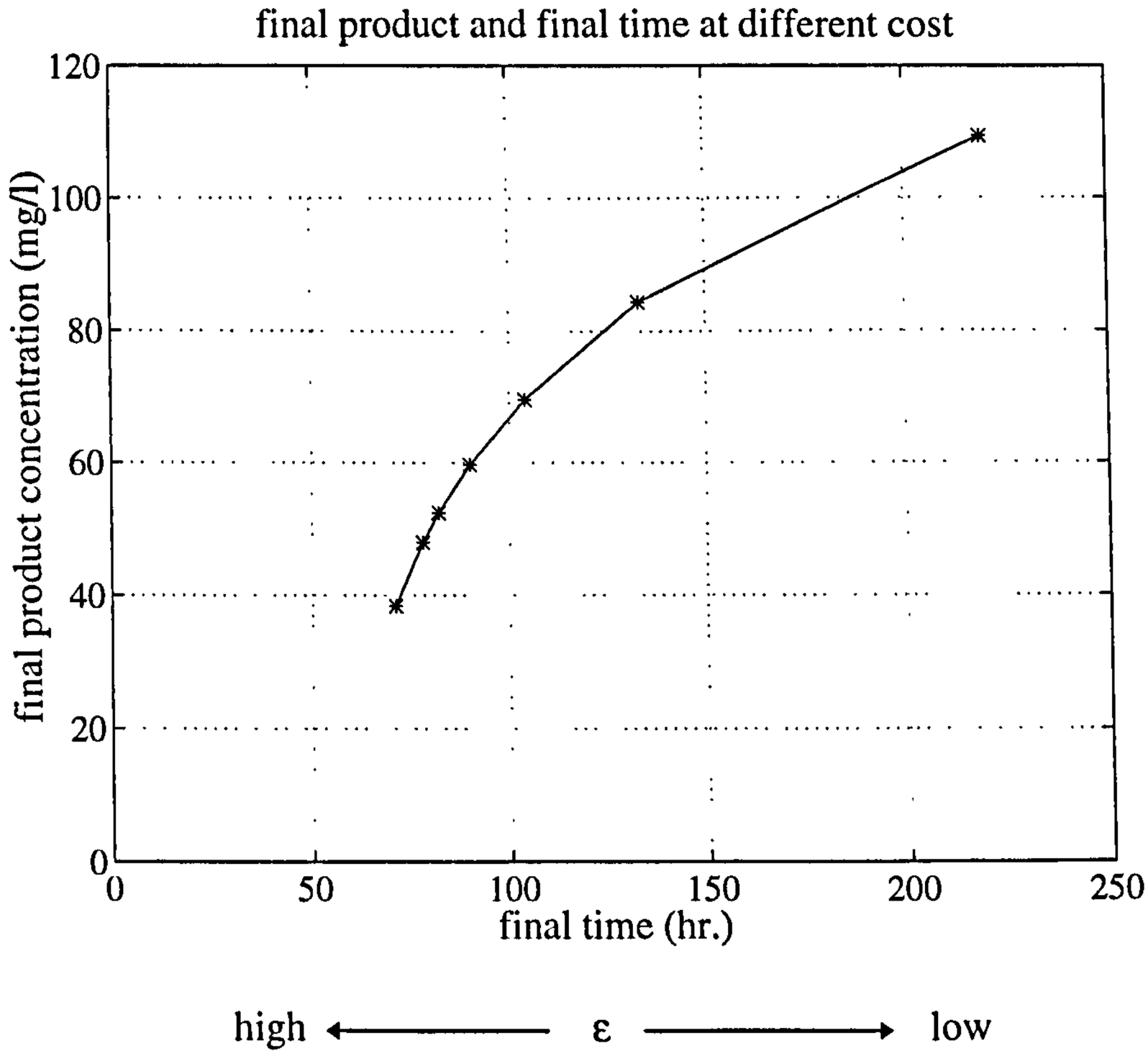


Figure 5-31 Relationship between maximum product and operating time at different cost factors

It is shown from the figure that as the cost factor increases, the operating time and maximum product decrease. This is due to the fact that the process is operated under a condition that increases the biomass growth rate and hence satisfies the increasingly importance of process operating time in the objective function. This is however sacrificed by the lower production in the process.

It is also worth mentioning that the graph shows the maximum product that can be obtained under the corresponding operating time. In this process, the possible shortest time

is when the cost factor is set at maximum and the process lasts for 71 hours The maximum product at this cost factor is 38.4 mg/l.

The operating time and product obtained from the OLOFP and CLOC methods under the condition of plant/model mismatch (refer to Table 5-6) are also plotted as shown in Figure 5-32. This is to show that it is not possible to get higher product than the optimal one under the similar operating time.

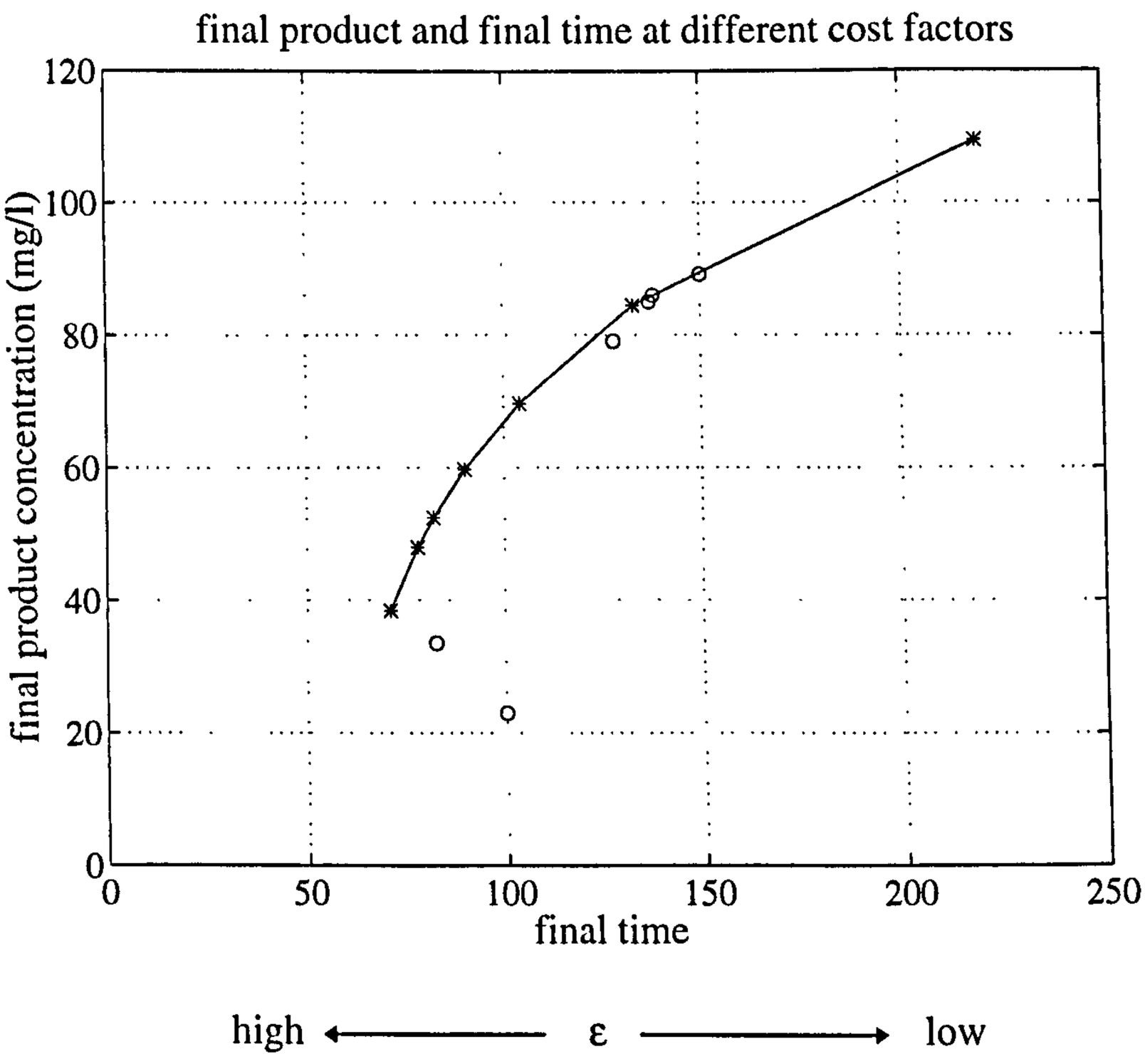


Figure 5-32 Relationship between final product and operating time length at different cost factors

*** refer to the optimal case with exact model**

o refer to the plant/model mismatch case

According to the figure, the highest maximum product that can be obtained from the process that does not operate at the optimal condition due to the plant/model mismatch is

equated to the optimal one. Note also that even this does happen, it was a result from the violating of cost factor in the objective function. For example, the maximum product for a pre-determined feed rate case, which the parameter Y_{xs} is 10% higher than the plant (refer to Table 5-6) is 89.14 mg/l at the length of operating time at 150 hours Although the maximum product is higher than the optimal one at this cost factor ($\varepsilon = 1$), the process takes longer operating time than the optimal one which is at 134 hours. The maximum product for this plant/model mismatch case, however, can not exceed the optimal curve in the figure (the co-ordinate of this mismatch example is at 150 hr and 89.14 mg/l in the figure).

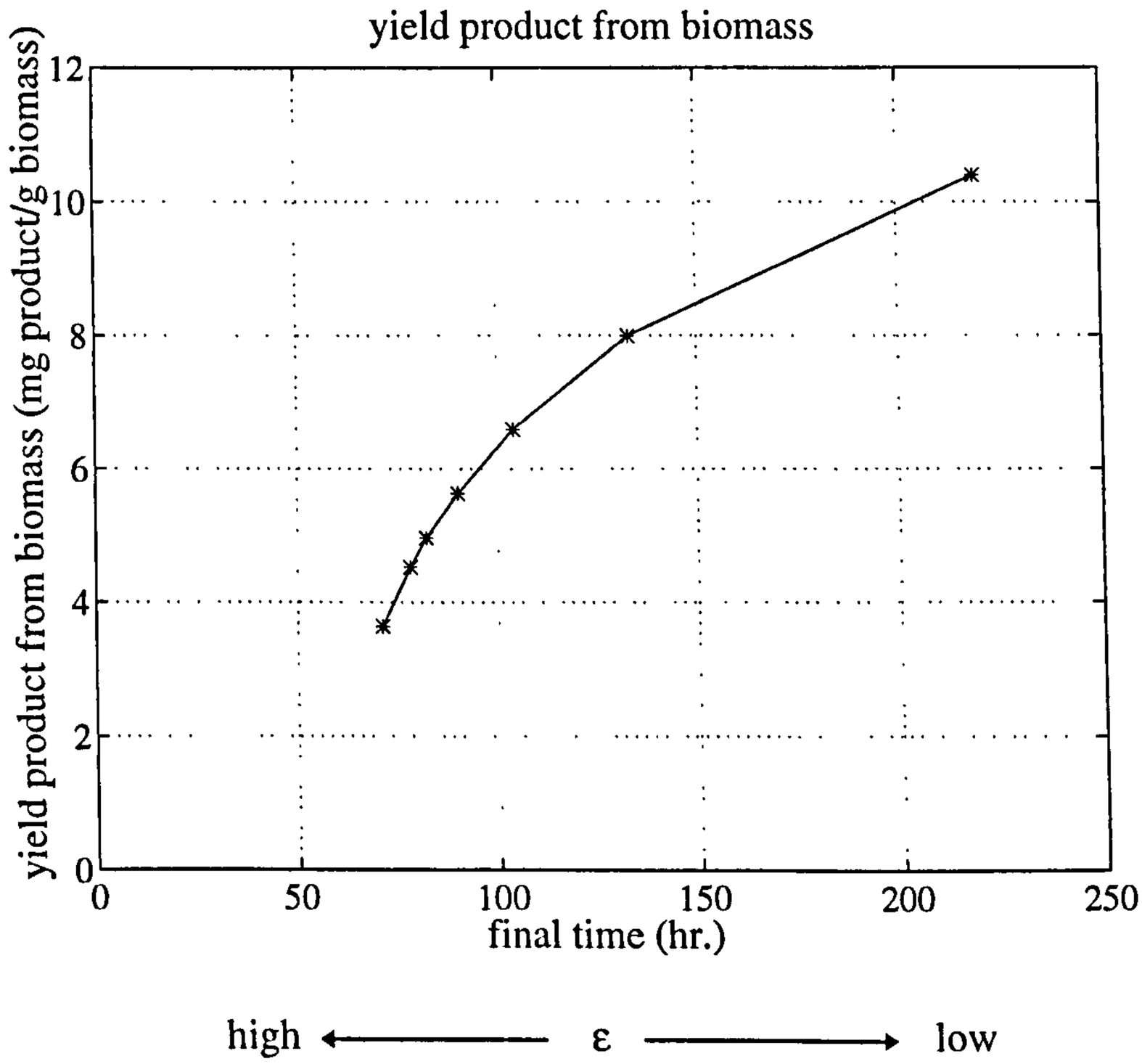


Figure 5-33 Relationship between Y_{px} and operating time at different cost factors

As the cost factor increases, the operating time and the yield of product from substrate decrease. The reason is the same as for the maximum product described earlier. Although this yield is not exactly the same as the ratio of π/μ, it has a very similar meaning.

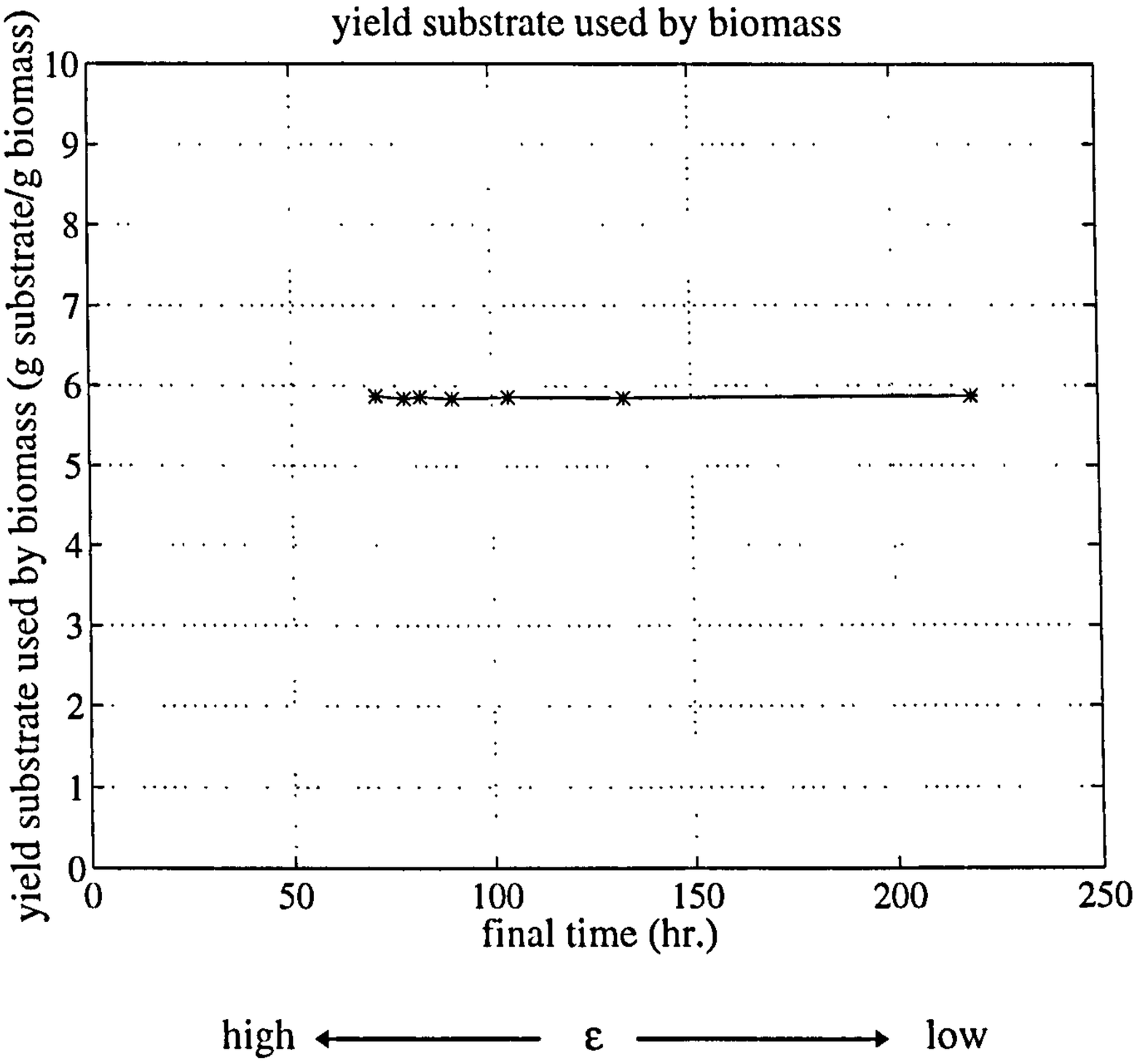


Figure 5-34 Relationship between Y_{sx} and operating time at different cost factors

From the figure, the yield of substrate consumed by biomass is constant. There is no effect of cost factor or operating time involved in this case since the same amount of substrate was added into the process and then converted into biomass with parameter Y_{xs} used in the simulation. As mentioned earlier, if Y_{sx} was determined by (5-20b), it would equal to the inversion of parameter Y_{xs}, which equals 6.1.

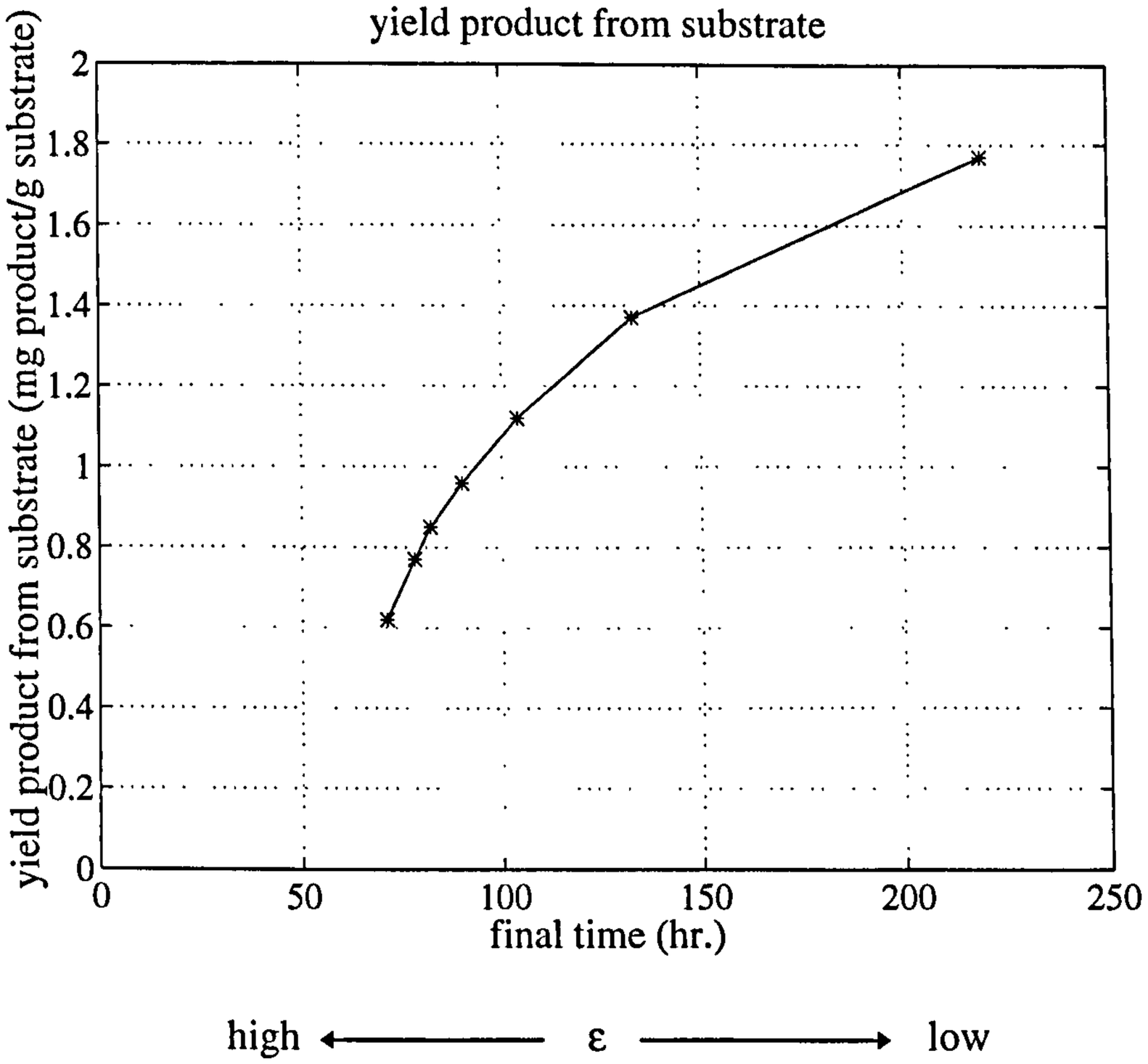


Figure 5-35 Relationship between Y_{ps} and operating time at different cost factors

Yield of product from substrate (Y_{ps}) is a proportion between Y_{px} and Y_{sx}. It shows the improvement of amount of product obtained under the longer operating time. It should be noted, however, that Y_{ps} used in here is only a generic term intended to show the improvement of production. It does not mean that at different conditions the amount of substrate that is transformed into a unit of product is different since this would violate the rule of material balance and therefore Y_{ps} in here does not mean the conversion rate of substrate to product. It shows however that at the different conditions, the micro-organisms activity on product formation is more emphasised than others and therefore more proportion of substrate is devoted for transforming into product which results in the higher yield (Y_{ps}).

5.2.3 Plant and Model Mismatch

It has been shown in the previous subsection that the proposed closed loop optimal control method has a benefit of feedback that makes it more superior than the open loop optimal feed rate profile method. For the controller part, both methods are actually the model-based controller. The singular feed rates (Equation (5-6) and (5-16)) both in case of pre-determined feed rate and nonlinear state feedback lack the ability to compensate the error in parameter Y_{xs} and S_f that are included in the singular feed rate calculation. For the CLOC method, the controller would attempt to track the optimal substrate profile trajectory while the error of parameter in the controller part is compensated by the feedback in the model predictive control scheme. Since the controller part is entirely separated from the optimal substrate concentration trajectory determination in the CLOC method, other types of controller can also be used particularly those base on robust control technique.

It has been considered only the plant/model mismatch on parameter Y_{xs} in this study. What is the effect of error in other parameters particularly those used in optimal substrate concentration determination ? and what are their effect on performance for both CLOC and OLOFP methods. This can be illustrated from considering the structures of the specific growth rate (μ) and specific product formation rate (π), as well as the singular feed rate and optimal substrate concentration profile for both primary and secondary metabolite production processes, which are shown in the following:

The specific growth rate:

$$\mu = \frac{\mu_{max} S}{K_s + S + S^2/K_i} \qquad (5\text{-}12)$$

The specific product formation rate:

$$\pi = \frac{\pi_{max} S}{K_{\pi s} + S + S^2 / K_{\pi i}} \tag{5-13}$$

Singular feed rate for a primary metabolite process:

$$F_{sing} = \frac{\mu X V}{Y_{xs}(S_f - S)} \; ; \; S = \sqrt{K_s K_i} \tag{5-6}$$

Singular feed rate for a secondary metabolite process:

$$F_{sing} = \frac{V}{(S_f - S)}\left(\frac{\mu X}{Y_{xs}} + \frac{\mu'(\pi'\mu - \pi\mu')}{(\pi'\mu'' - \pi''\mu')}\right) \tag{5-16}$$

Optimal substrate concentration for a primary metabolite process:

$$S_{opt} = \sqrt{K_s K_i} \tag{4-16}$$

Optimal substrate concentration for a secondary metabolite process:

$$\dot{S} = -\frac{\mu'(\pi'\mu - \pi\mu')}{(\mu'\pi'' - \pi'\mu'')} \tag{5-14}$$

Considering the singular feed rate and the optimal substrate concentration trajectory, both have the specific reaction rates (μ, π) and their derivatives (μ', μ'', π', π'') in common in both primary and secondary metabolite processes. Since the optimal substrate trajectories are determined from these reaction rate and their derivatives, error in these parameters - μ_{max}, K_s, K_i, π_{max}, $K_{\pi s}$ and $K_{\pi i}$ would result in the incorrect optimal substrate concentration profile. Although both the CLOC and OLOFP methods would give the non optimal substrate concentration profile and feed rate, the feedback property of the CLOC method is still proved to be useful as shown in the following example of a biomass production, in which parameter K_s in the model is incorrect.

In this illustration, it is assumed that parameter K_s in the model equals 3, while the real parameter in the process equals 2 and 4. The simulation results for both OLOFP and CLOC methods in case of higher and lower incorrect parameters are shown in Figure 5-36 to Figure 5-39.

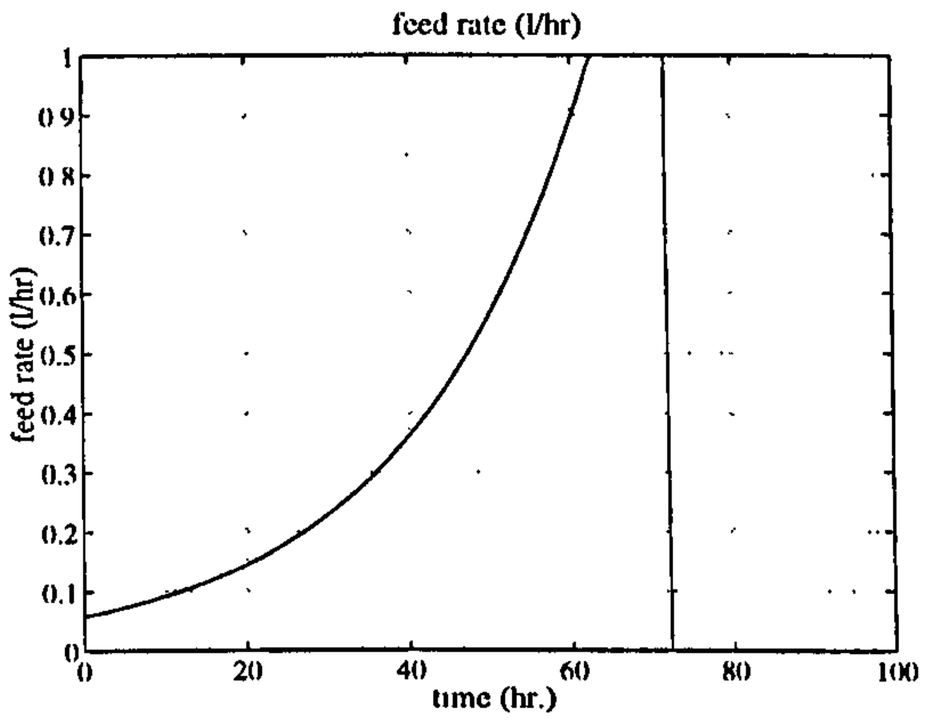
For the OLOFP method, the optimal feed rate is determined based on parameter K_s equals 3 and should maintain the substrate concentration in the fermenter at 5 g/l if the model is correct. The simulation results in Figure 5-36 in which the true process parameter K_s equals 2 show that with the pre-determined feed rate, the substrate concentration deviates from the optimal substrate concentration that equals 4.083 g/l ($S_{opt} = \sqrt{K_S K_i}$). Also, due to the incorrect parameter, the substrate concentration can not be kept at 5 g/l either. In case of parameter K_s equals 4, the true optimal substrate concentration equals 5.774 g/l. The simulation results in Figure 5-37 also show that the substrate concentration deviates from the optimal level due to the incorrect parameter in the model. Decreasing of the substrate concentration from 5 g/l to zero in Figure 5-36 due to the fact that the real process has a higher specific growth rate (Figure 5-36 (d)) than that calculated from the model. This results in micro-organisms being produced at faster rate than expected from the model. Therefore the pre-determined feed rate can not provide enough amount of substrate to maintain the substrate concentration in the fermenter at 5 g/l as calculated from the model. The same reason also applies for increasing of the substrate concentration in Figure 5-37. The specific growth rate in this process ($K_s = 4$) is lower than that calculated from the model (Figure 5-37 (d)). This results in micro-organisms being produced at slower rate than expected. Therefore, the pre-determined feed rate provides higher amount of the substrate concentration than needed and resulting in the increasing of

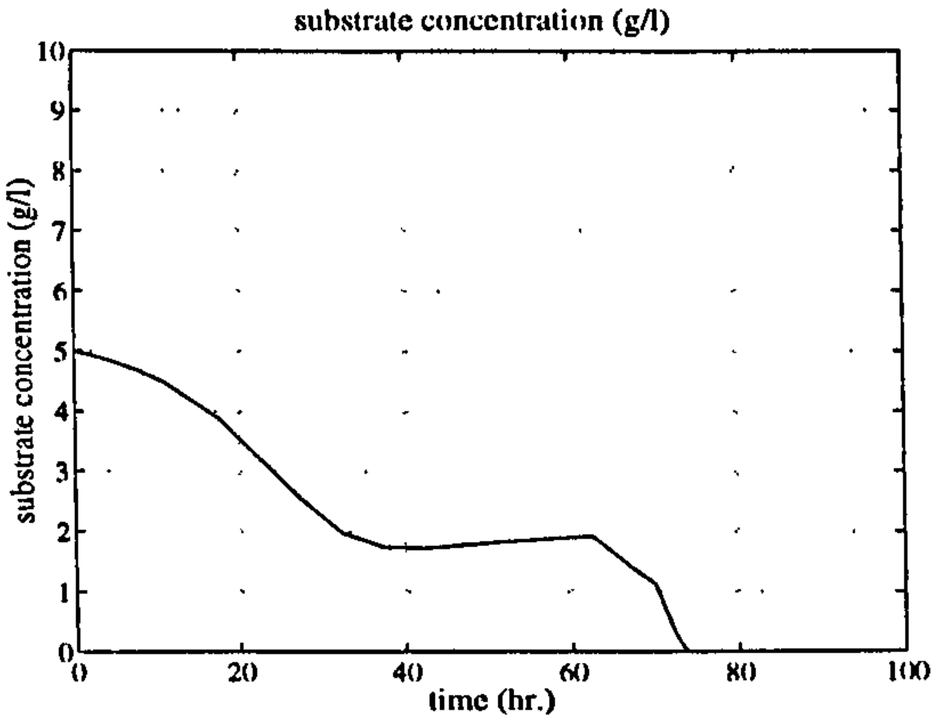
substrate concentration. In the figure, the substrate concentration starts decreasing after the substrate feed rate has stopped.

For the CLOC method, the substrate concentration is kept at 5 g/l (The optimal substrate concentration that is calculated from the model). Although the true optimal levels are 4.083 g/l and 5.774 g/l (for the process with K_s equals 2 and 4 respectively), keeping the substrate concentration at 5 g/l has a little effect to the process operating time comparing with the OLOFP method. As shown in (d) in Figure 5-36 and Figure 5-37, keeping substrate concentration at 5 g/l results in slightly lower maximum specific growth rate than keeping it at 4.083 g/l and 5.774 g/l (the optimal substrate concentrations). The process operating time for both methods in each case is shown in Table 5-9.

It is shown in the table that for the incorrect parameter as shown in the simulation (Figure 5-36 to Figure 5-39), the CLOC method give a better performance than the OLOFP method. For the process with K_s =2, the operating time if the parameter in the model is correct is 68 hours. The operating time for the CLOC method is 68.5 hours, which is a little longer than the true one and shorter than that obtained from the OLOFP method, which is at 74 hours. For the process with K_s = 4, the operating time if the parameter in the model is correct is 81 hours. The operating time for the CLOC method is 82 hours and that obtained from the OLOFP method is 97 hours.

This illustration shows the advantage of the CLOC method over the OLOFP method even in the case of incorrect parameter that is used in the optimal substrate concentration determination.





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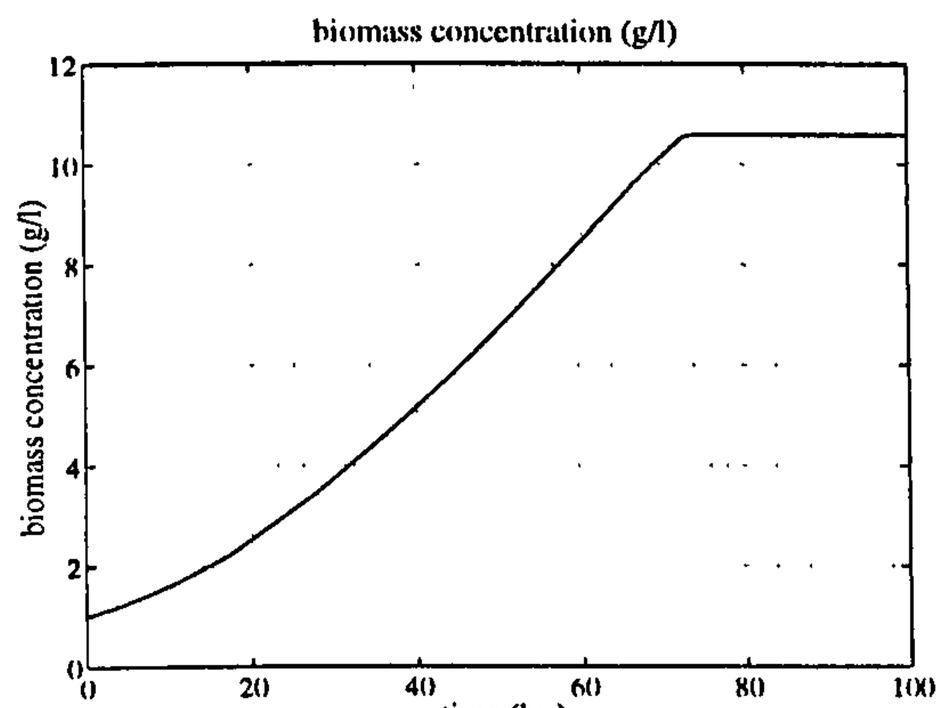

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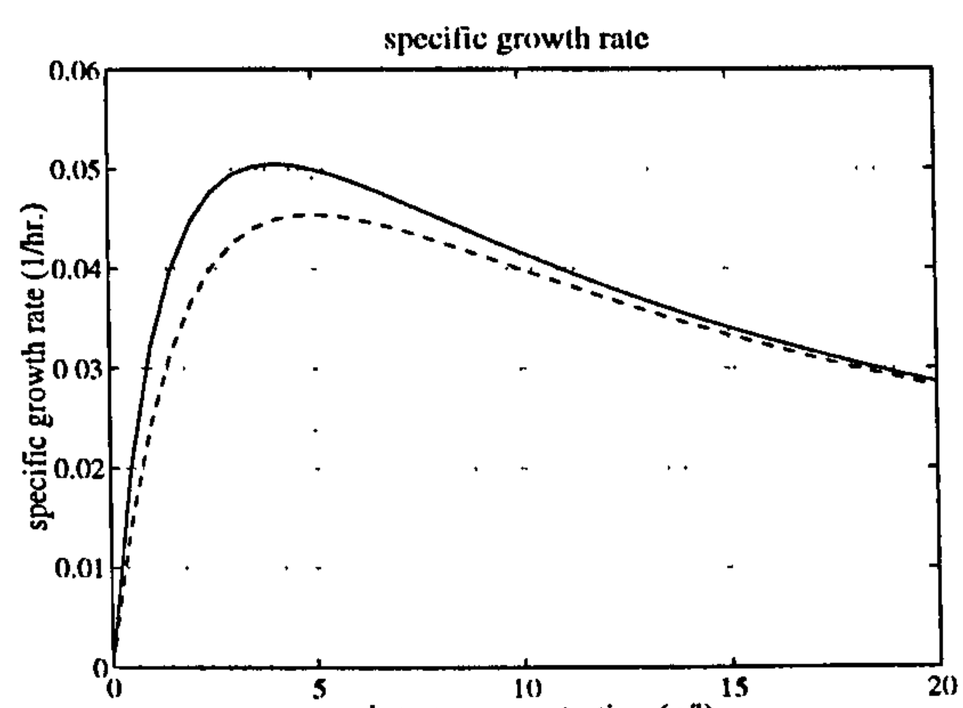


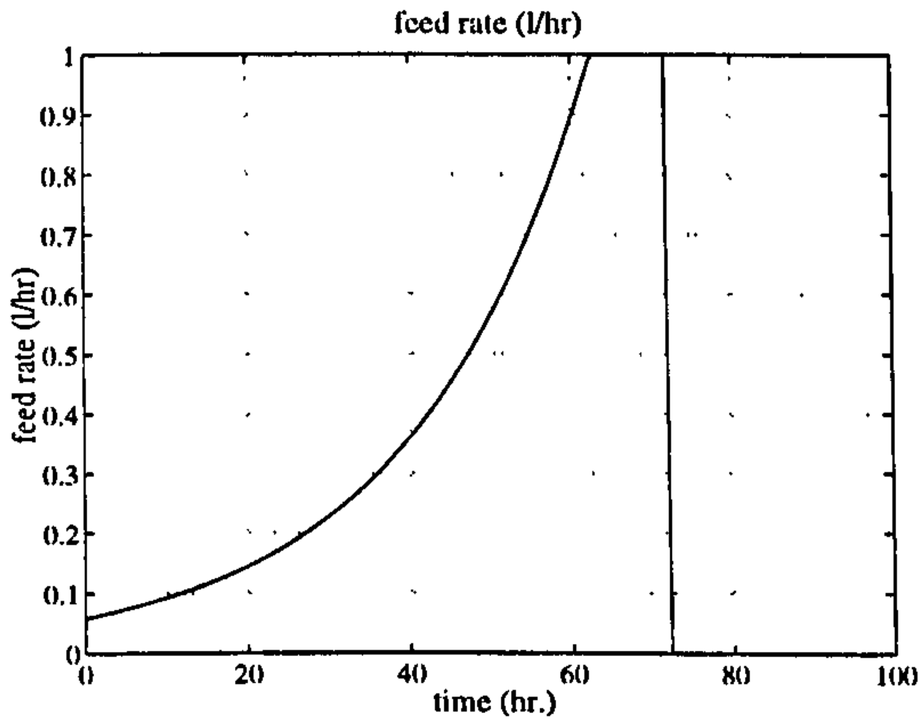
(c)

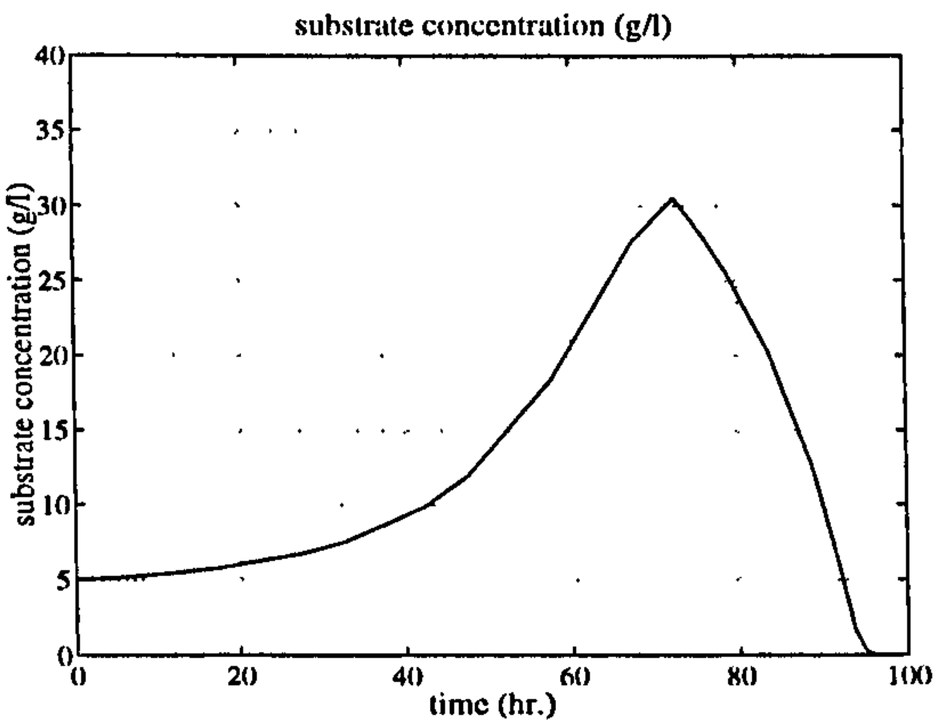
(d)

Figure 5-36 Simulation of a primary metabolite production - incorrect parameter for the OLOFP method (true $K_s = 2$, model $K_s = 3$)

Solid line in (d): specific growth rate with $K_s = 2$

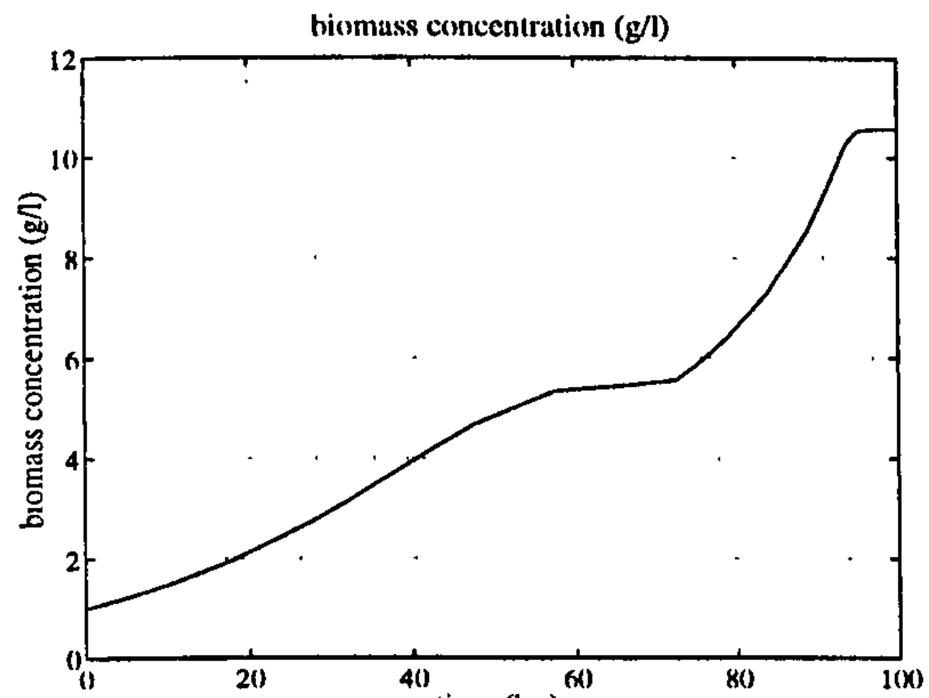
Dashed line in (d): specific growth rate with $K_s = 3$

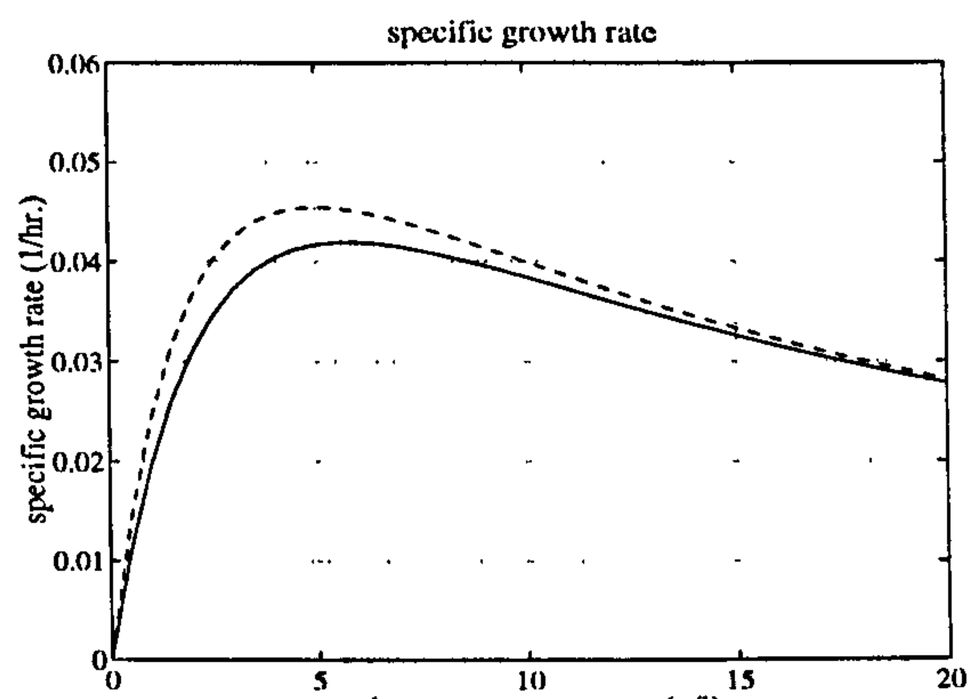




(a)

(b)





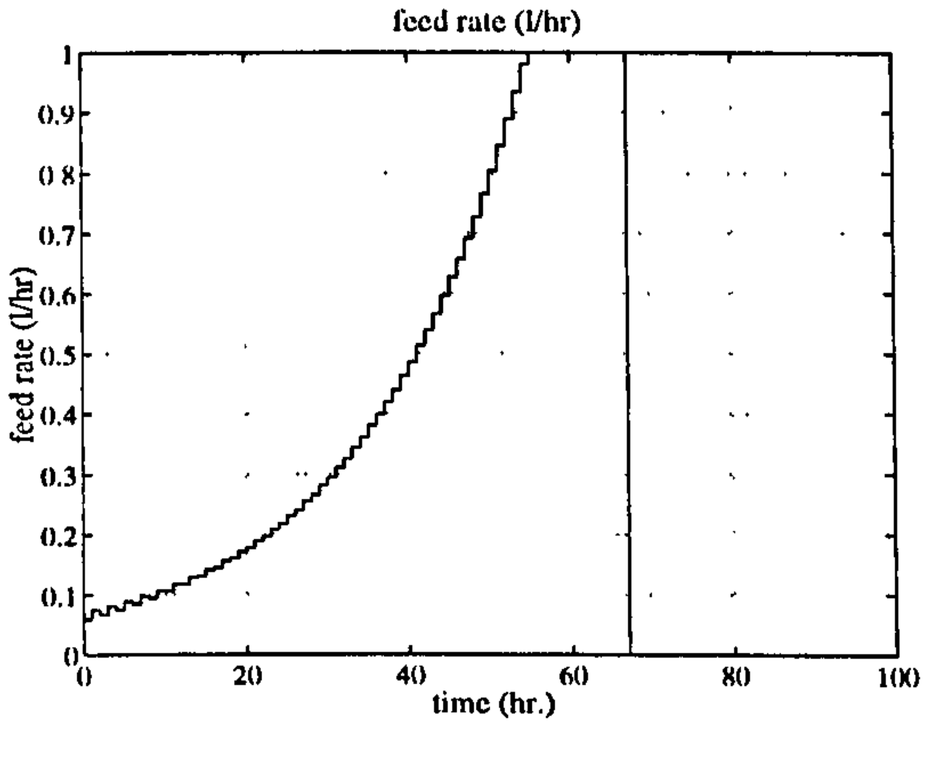
(c)

(d)

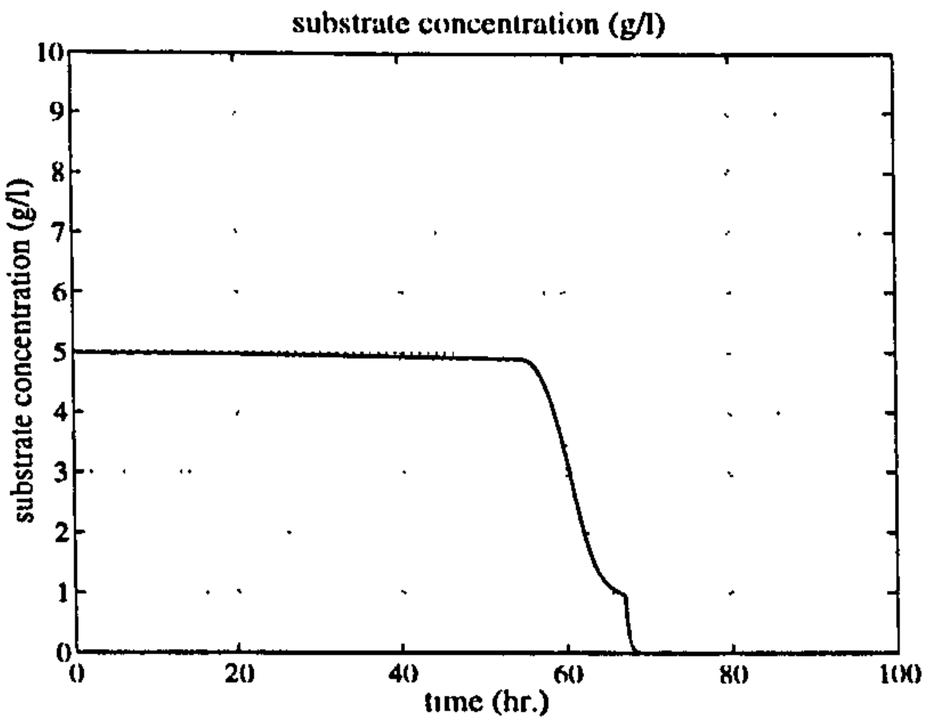
Figure 5-37 Simulation of a primary metabolite production - incorrect parameter for the OLOFP method (true $K_s = 4$, model $K_s = 3$)

Solid line in (d): specific growth rate with $K_s = 4$

Dashed line in (d): specific growth rate with $K_s = 3$



(a)



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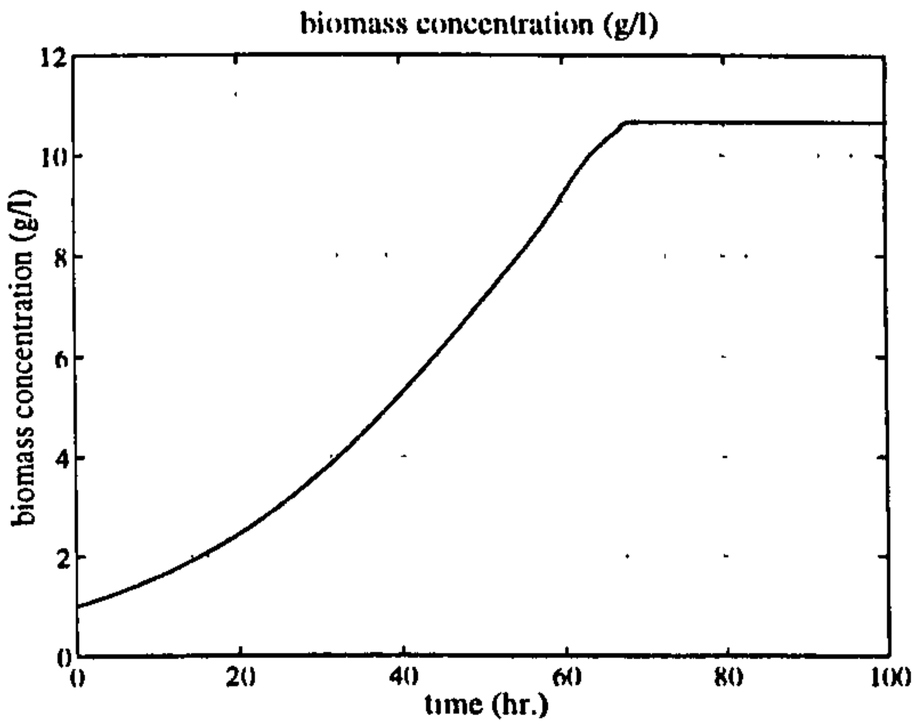
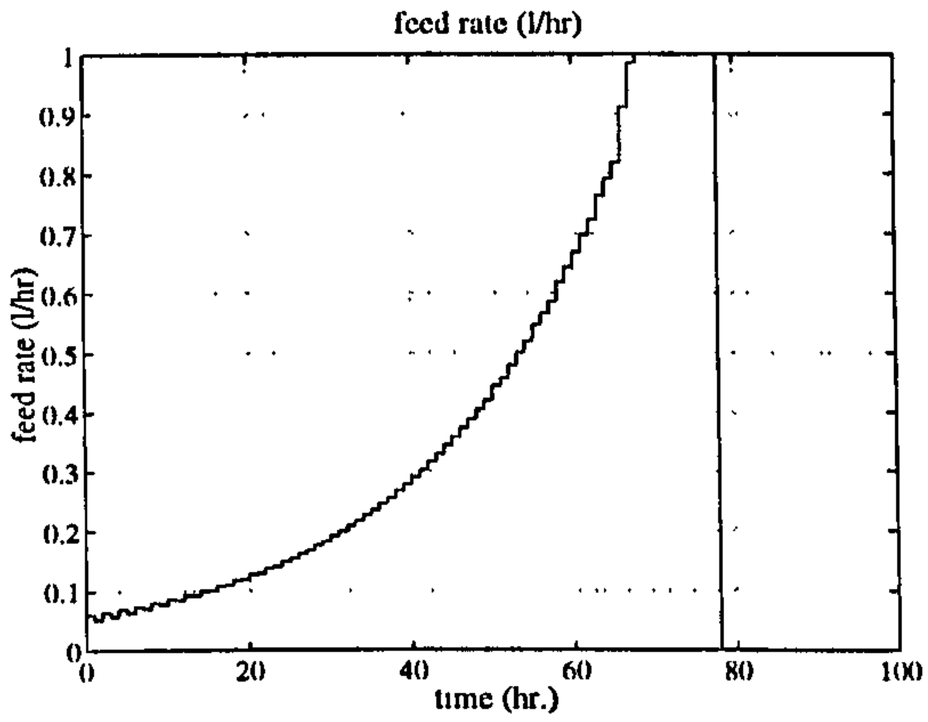
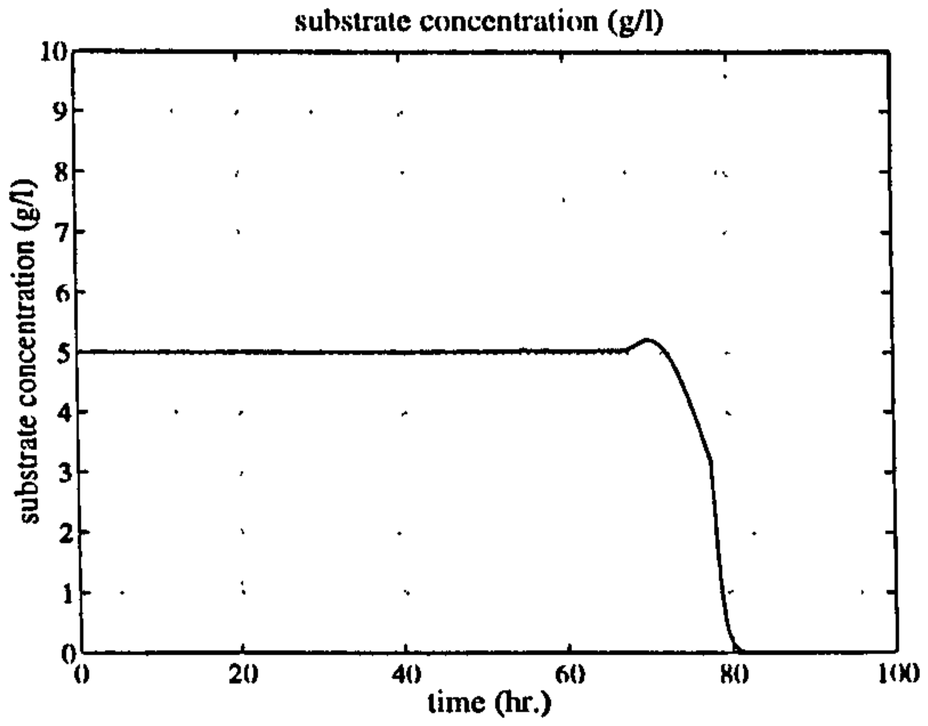


(c)

Figure 5-38 Simulation of a primary metabolite production - incorrect parameter for the CLOC method (true $K_s = 2$, model $K_s = 3$)



(a)



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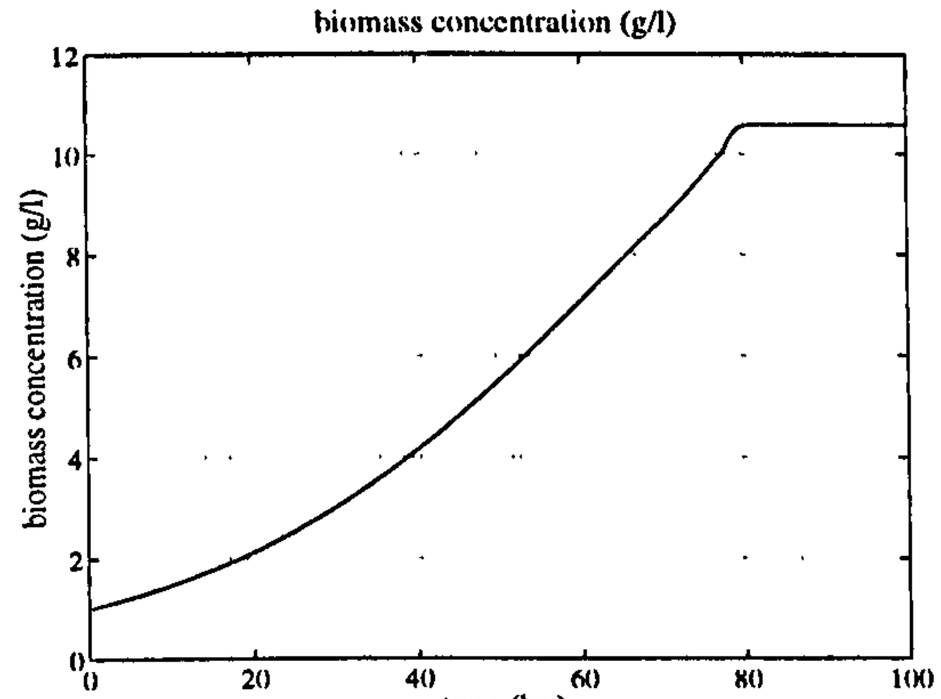

(c)

Figure 5-39 Simulation of a primary metabolite production - incorrect parameter for the CLOC method (true $K_s = 4$, model $K_s = 3$)

Table 5-9 Effect of incorrect parameter K_s to the process operating time

Correct parameter K_s	Operating time (hr.)		
	Correct parameter for both methods	Incorrect parameter (K_s = 3)	
		CLOC	OLOFP
2 (-50%)	68	68.5	74
4 (25%)	81	82	97

In the table, the correct process parameter K_s is assumed to be at 2 and 4 while the incorrect model parameter K_s is 3. The percentage of parameter variation is then calculated as:

For Ks = 2, the parameter variation is $\frac{2 - 3}{2} * 100 = -50\ \%$

For Ks = 4, the parameter variation is $\frac{4 - 3}{4} * 100 = 25\ \%$

The process operating time in the table shows the advantage of the CLOC method over the OLOFP method even in the case of incorrect parameter that is used in the optimal substrate concentration determination.

Chapter 6 Conclusion and Further Work

The calculus of variations method has traditionally been used for optimisation of fed-batch fermentation processes. Since the method usually needs an iterative calculation to get an answer, the resulting optimal control strategy is therefore pre-determined off-line and performs in an open loop manner. There are some specific cases where the optimal control can be calculated for closed loop implementation. An example is a linear quadratic optimal control, in which the system is linear and the objective function is in a quadratic form.

The optimal feed rate profile usually derived in the literature consists of a combination of maximum, minimum and singular feed rates. It has been shown in this study that the singular feed rate was used to maintain the substrate concentration at an optimal level, which optimises a given objective function. This coincides with a knowledge of the process since the bioreaction rates are governed by the substrate concentration. Many industrial processes also maintain the substrate concentration at a constant level, which is known to be suitable for microbial production of desired products. However, the constant substrate concentration level might not be optimal for the whole batch particularly for a secondary metabolite process in which conditions suitable for microbial growth and secondary product formation are different. This raises the need for the development of optimal substrate profiles especially as a reliable (neural network-based) on-line estimation of substrate concentration is now available (Zhang, *et al.*, 1996). The following two step optimisation method of fed-batch fermentation processes has therefore been developed in this research. The proposed method divides an optimisation problem in the fed-batch fermentation into two parts.

1. Determination of an optimal substrate concentration profile, which optimises a given objective function.

2. Designing a controller to track the obtained substrate concentration profile.

The proposed method operates the system in a closed loop manner and is therefore called "closed loop optimal control". The Closed Loop Optimal Control (CLOC) and the Open Loop Optimal Feed rate Profile (OLOFP), which is used in the literature are shown to have a very close relationship, particularly the similar pattern of substrate feed rate and optimal substrate concentration profile. Although the substrate concentration profile obtained after applying the pre-determined optimal feed rate is similar to the one obtained by the CLOC method, this information on optimal substrate concentration profile is not explicitly shown to the operator under the OLOFP scheme.

The main advantages of the closed loop optimal control strategy compared to the open loop optimal feed rate profile approach are two fold:

1. The closed loop optimal control strategy can avoid a singular control problem that happens in the open loop optimal feed rate profile method since the substrate concentration appears nonlinearly in the system equation as well as the Hamiltonian.

2. The control problem is converted into a closed loop control that can be expected to perform more robustly than an open loop control.

Simulations on primary and secondary metabolite processes have been performed to compare these two methods. For the comparison simulation, all the specific bioreaction rates are functions of substrate concentration as we used substrate feed rate to manipulate the fed-batch fermentation. The specific substrate usage rate (σ) is a proportion of the specific growth rate. The specific growth rate (μ) and specific product formation rate (π)

are in a substrate inhibition kinetic form. The Monod type kinetic has not been used for the simulation because the fermentation process with this kinetic will be suitable to operate the fermentation in batch mode. The simulation results can be summarised as follows;

- For a primary metabolite production process, the substrate concentration is kept at a fixed level, which maximises the specific growth rate. A biomass production process is used as an example for the primary metabolite process. Since the substrate concentration is kept at the level, which maximises the specific growth rate, the maximum biomass can be obtained at the shortest operating time.

- For a secondary metabolite production process, the secondary metabolite production depends not only on the substrate concentration level that maximises the specific product formation rate but also on the amount of biomass in the fermenter. Two cases are therefore considered. For an objective function, which does not include the cost of operating time, the substrate concentration would be kept at the constant level, which maximises the ratio between the specific product formation rate (π) and the specific growth rate (μ). This results in maximum product at the end of the batch but with the expense of long operating time. In the other case where an objective function includes the cost of operating time, the optimal substrate concentration is not constant but follows an optimal profile. Since the optimal substrate concentration profile is only a function of the substrate concentration, the different cost factors (ε) take effect on different relations between biomass and substrate concentration that provoke the optimal substrate concentration profile.

- For a perfect model case, the CLOC and OLOFP methods give similar results. The constraints on feed rate and culture volume is taken care of within the variational method and the Pontryagin's maximum principle in the OLOFP method while in the

CLOC method, these constraints are accommodated as part of the model predictive control.

- For an imperfect model case, the CLOC method provides better performance than the OLOFP method. For the incorrect model parameters that involve in calculating substrate feed rate, the better performance is due to the fact that the error in the parameters (for example, Y_{xs}) or disturbance in substrate feed rate concentration (S_f) are automatically compensated for by feedback in the CLOC method. In case of the incorrect model parameters that are used for determining the optimal substrate concentration profile (μ_{max}, K_s, K_i, π_{max}, $K_{\pi s}$ and $K_{\pi i}$), The CLOC method still provides the better performance than the CLOFP methods. As the controller is used for tracking the optimal substrate concentration profile in the CLOC case, the choices of controller are also widely open. Several types of controller can be used including robust controllers for dealing specifically with the model uncertainty.

The proposed method can also be extended to other environmental variables that have effects on the bioreaction rate. These variables are, for example, DOT, temperature and pH. For a combination of these variables, the optimal solution might emerge as an optimal surface of these variables. The system then becomes a multi-input and multi-output one. The overall scheme can also be extended to cover other factors in the objective function as shown in Figure 6-1.

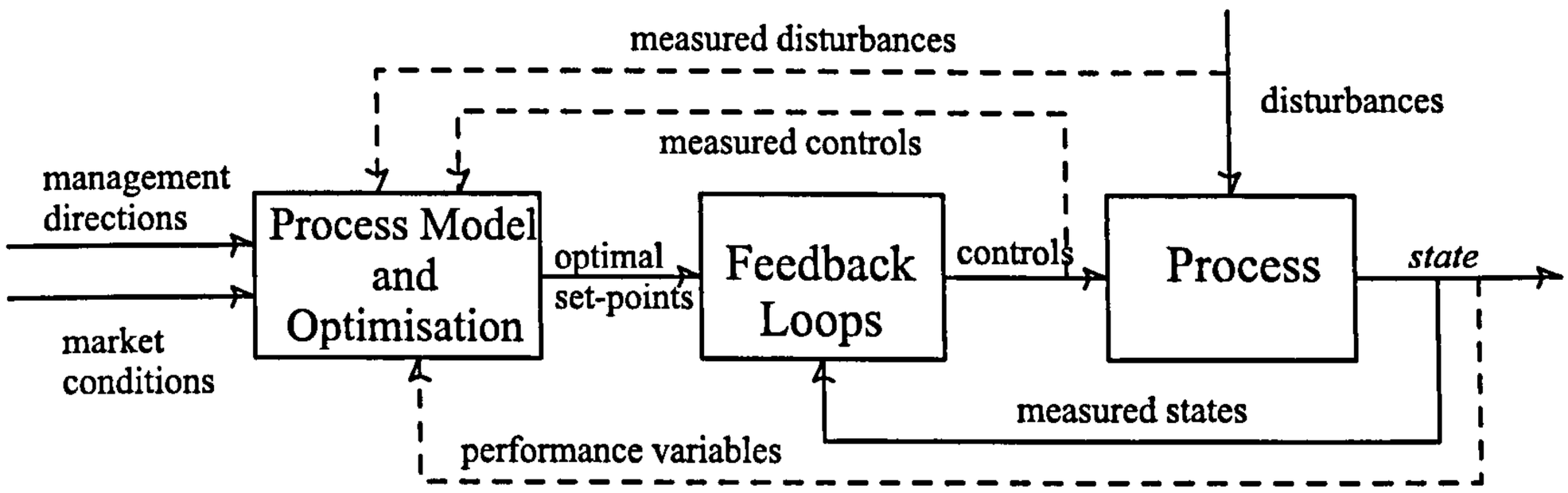

Figure 6-1 General diagram of the CLOC method

The material in this thesis has established the relationship between the open loop optimal feed rate control and closed loop optimal control of fed-batch fermentation processes. The advantages of the closed loop optimal control have also been shown. However, there is more work remains to be undertaken to take into account other aspects that are needed for implementation of the closed loop optimal control method and improving the overall optimisation of the fermentation processes. This work includes the following:

- Improvement of the process model. This requirement can be seen clearly from the optimisation and controller design point of view on the accuracy of process model. The calculus of variations used in this thesis for optimising the fed-batch fermentation processes is also based heavily on the process model. Although the proposed method can improve the performance on the case of incorrect model parameter compared with the conventional open loop method, the improvement is still limited. A lot of developments on modelling are needed especially on the structured model despite the fact that this type of model has not been used for the optimising of fermentation processes (Johnson, 1987). Metabolism understanding and advances in modelling of metabolic network inside the micro-organism (Delgado and Liao, 1992; Geraats, *et al.*,

1990; Kell, *et al.*, 1989; Liao and Delgado, 1992; Nielsen and Jorgensen, 1995; Rizzi, *et al.*, 1995; Shimizu, *et al.*, 1995; Wiechert, *et al.*, 1995) will also play an important role in the future of process optimisation. As the computational power of modern computers increases dramatically in the past few years, complicated model with numerous parameters for the optimisation purposes will be easily implemented in the near future.

- Optimal experimentation. As the modelling procedure needs data from experiments, the data should contain as much information as possible. The planning of experiments plays an important role especially for the fermentation processes as each experiment is usually time consuming and costly. Many experimental methods such as factorial design and response surface analysis (Box, *et al.*, 1978) are usually based on static experiments in which the dynamics of the process is not considered. Examples of this type of experimental design to improve the fermentation processes are in (Banerjee and Bhattacharyya, 1993; Chen, 1994; Reinikainen, *et al.*, 1985). Much work on optimal experimental design in dynamic mode are usually emphasised on improving the accuracy of model parameters and on discriminating between different model structures. The criteria are based on evaluation of the information matrix. (Cobelli and Thomaseth, 1985; Cooney and McDonald, 1995; Giladi and Sideman, 1989; Hass and Munack, 1990; Johnson and Berthouex, 1975a; Johnson and Berthouex, 1975b; Munack, 1989; Munack and Posten, 1989; Vanichsriratana, *et al.*, 1993; Vialas, *et al.*, 1985; Yoo, *et al.*, 1986). Although the optimal experimental design of dynamical system and optimal input signal for system identification have been studied in the control literature for more than two decades (Aoki and Staley, 1970; Arimoto and Kimura, 1971; Godfrey, 1993; Goodwin, 1969; Goodwin, 1971; Goodwin and Payne,

1973; Goodwin and Payne, 1977; Goodwin, *et al.*, 1974; Mehra, 1974; Zarrop, 1979), the application of this approach into fermentation processes has just started and many questions are still waiting for the answers. For example, what are suitable magnitudes and frequencies for the excitation signals ? This topic is therefore one of the most important tasks to achieve the accurate modelling.

- On-line measurement of state variables and on-line model updating. The optimisation method used in this thesis needs feedback from the measurement of substrate concentration. Substrate concentration and other state variables such as biomass and product concentration are not usually on-line measurable. These measurements are usually done by analytical assay in laboratory, which may take many minutes up to hours for analysing one sample. This results in time delay of the feedback in the process. The number of samples are also limited by the high analytical cost. There are many attempts to overcome this problem. One of them is biosensors. The development of biosensors however still does not yet overcome the problem of heat and stability from the process sterilisation as well as the measurement specificity and interference of other biochemical compounds in fermentation culture. Hence it is not yet truly applicable in industry. There is also an effort to integrate analytical equipment into the process such as on-line HPLC (High Performance Liquid Chromatography) (Saucedo, *et al.*, 1995) and FIA (Flow Injection Analysis) (Hitzmann, *et al.*, 1995). However, these equipments still need several minutes for analysing each sample. The variation in analytical time depends on the substance being analysed. Although there is a lot of improvement from the analysis in the laboratory, the limited number of samples and long time delay might still confine the implementation of advanced control techniques and also the optimisation in this case. Another method is to use a state estimation

technique. This method is based on the Kalman or extended Kalman filter to estimate state variables from the on-line measurement such as OUR or CER. The literature in this application to fermentation processes is tremendous (Aborhey and Williamson, 1978; Charbonnier and Cheruy, 1994; Dekkers, 1982; Dochain, *et al.*, 1989; Flaus, *et al.*, 1991; Ghoul, *et al.*, 1985; Grosz, *et al.*, 1984; Heijden, *et al.*, 1989; Liu, *et al.*, 1992; Mirzai, *et al.*, 1990; Nahlik and Burianec, 1988; Park, *et al.*, 1983; Ramirez, 1987; San and Stephanopoulos, 1984a; San and Stephanopoulos, 1984b; Stephanopoulos and San, 1981; Stephanopoulos and San, 1984; Thatipamala, *et al.*, 1993; Tsao, *et al.*, 1991), although none appears in the recent 6th -ICAB conference. Recently, a reliable estimation of substrate concentration based on an artificial neural network has been successfully developed and operates at Pfizer for the Oxytetracycline production plant (Zhang, *et al.*, 1996). As state estimation is a current method that can provide on-line estimation of state variables, the integration of this estimation into the proposed optimisation scheme should be investigated and the effect of the estimation to the optimisation performance should be quantified. Figure 6-2 shows the diagram that integrates the state estimation into the process optimisation scheme. The diagram also shows the possibility for the model to be updated from the available measurements. When the process model has been updated, a new optimal substrate concentration profile is generated. The optimality on incorporation of the new profile and the corresponding control action in this system will need further investigation. The notation in the diagram has the following meaning: y are on-line measurements, such as OUR and CER, x are state variables, such as biomass, substrate and product concentration and $\hat{x}$ are on-line estimated state variables.

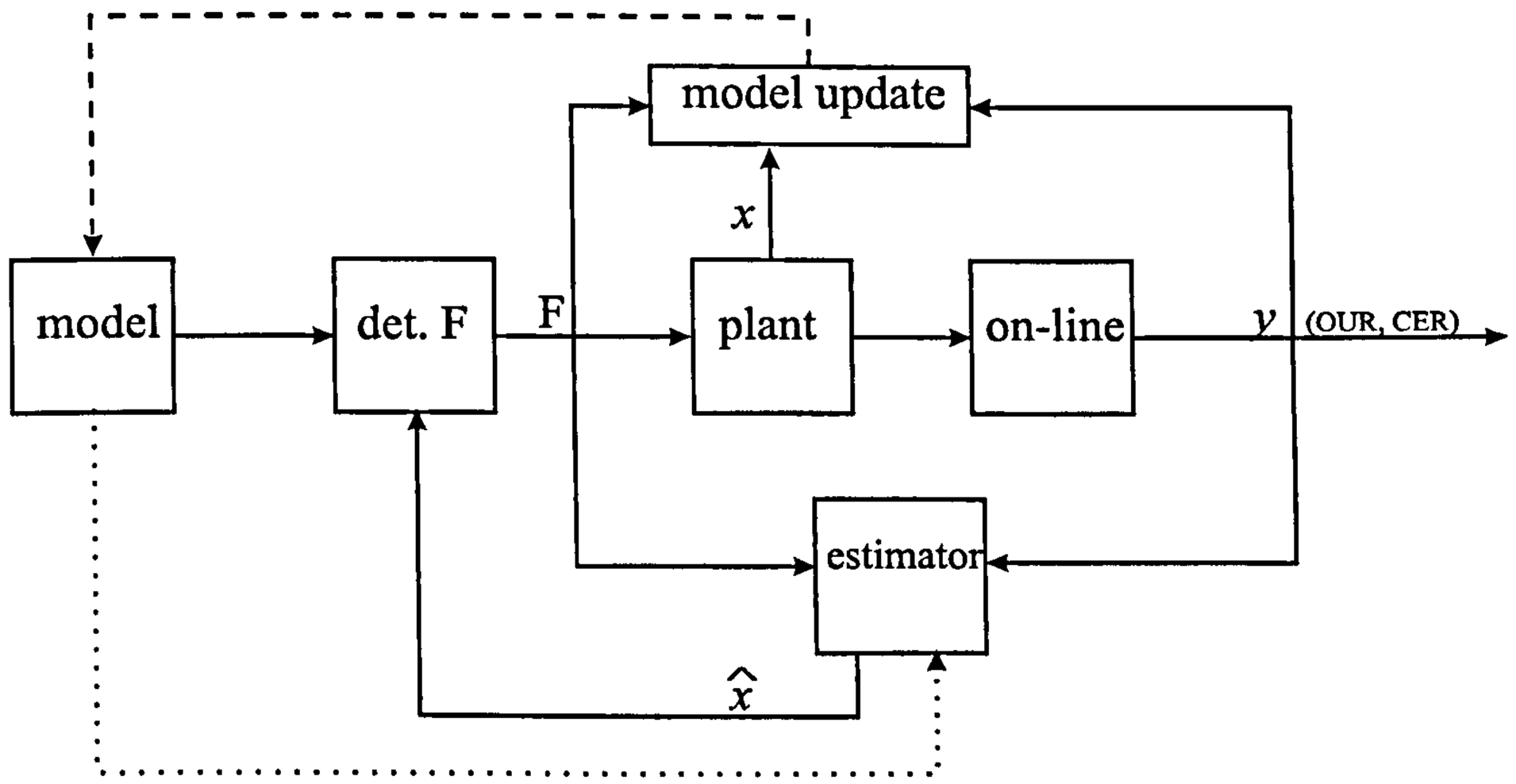

Figure 6-2 Diagram of a state estimation integrated into the optimisation scheme

- On-line optimisation. In some processes, where process models are difficult to obtain or inaccurate, an on-line optimisation might be necessary. This direction would be another practical step forward in the optimisation of fermentation. A typical on-line optimisation scheme is shown in Figure 6-3 (Chang and Lim, 1989; Chang and Lim, 1990; Chang, *et al.*, 1988; Hamer and Richenberg, 1988; Hilaly, *et al.*, 1994; Rolf and Lim, 1984; Rolf and Lim, 1985; Semones and Lim, 1989).

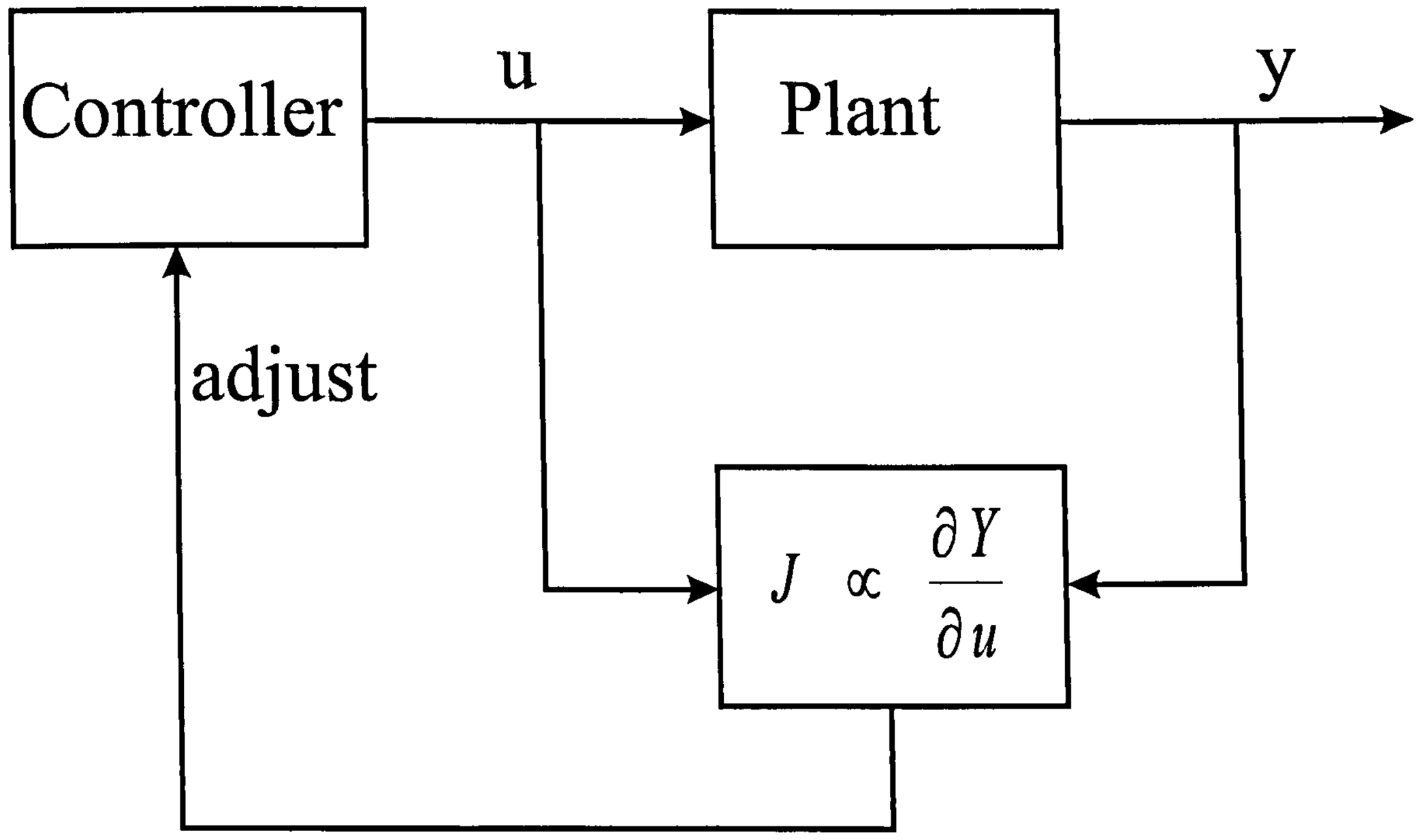

Figure 6-3 Diagram of an on-line optimisation scheme

In figure 6-3, u is referred to as feed rate while y is product concentration. The current gradient between u and y is used to calculate the next move of u. As the substrate concentration has an effect on product formation, the following scheme shown in Figure 6-4 should also be investigated. In the figure, x is referred to as substrate concentration. The current gradient between y and x provides the next optimal value of substrate concentration and the feed rate (u) can then be calculated.

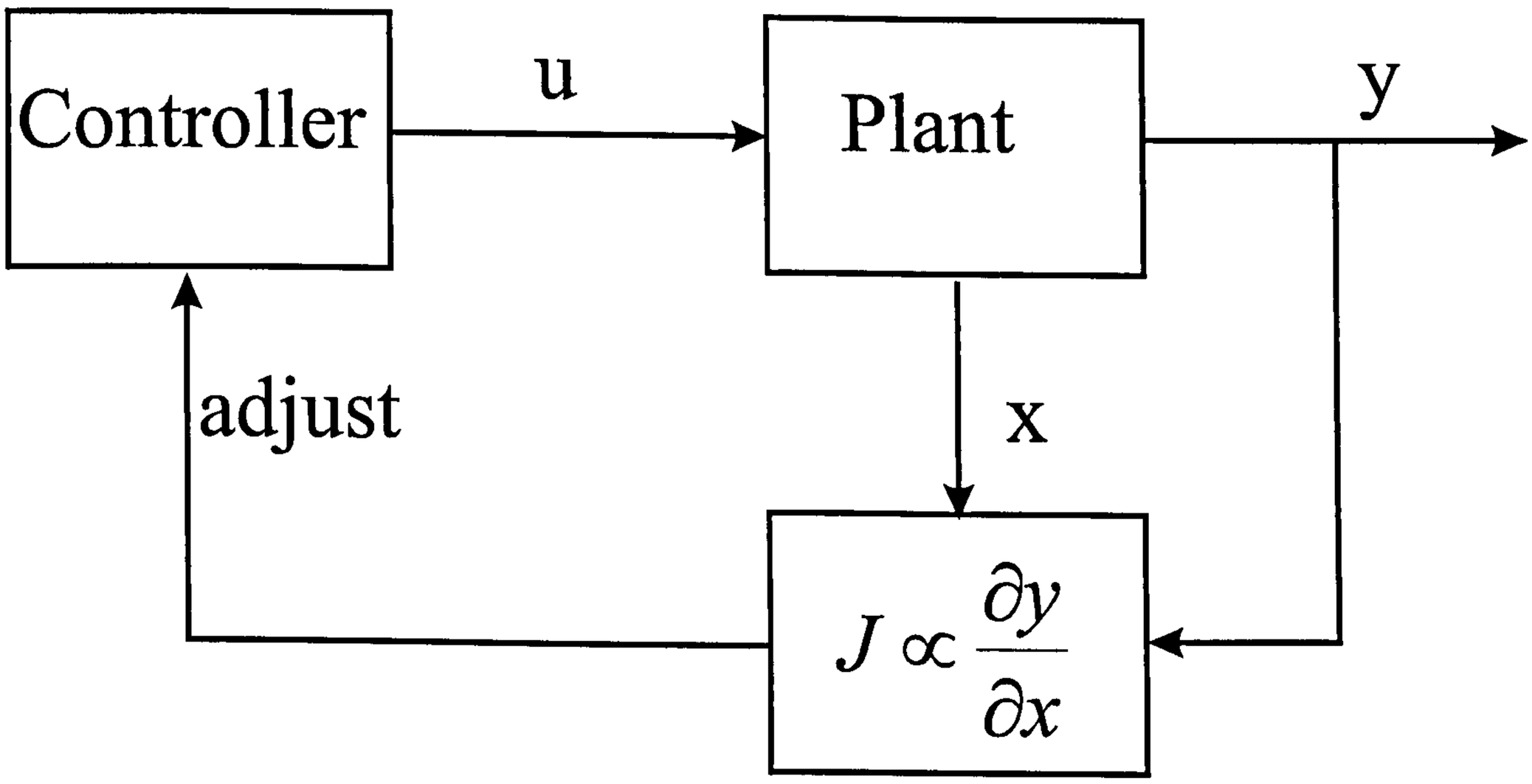

Figure 6-4 Diagram of an on-line optimisation scheme based on the environmental parameters

References

Aborhey, S. and Williamson, D. (1978). State and Parameter Estimation of Microbial Growth Processes. *Automatica*, **14**, 493-498.

Agrawal, P., Koshy, G. and Ramseier, M. (1989). An Algorithm for Operating a Fed-Batch Fermentor at Optimum Specific Growth Rate. *Biotech.Bioeng.*, **33**, 115-125.

Ali, E. and Zafiriou, E. (1993). Optimization-based Tuning of Nonlinear Model Predictive Control with State Estimation. *J.Proc.Cont.*, **3**(2), 97-107.

Anderson, B. D. O. and Moore, J. B. (1989). *Optimal Control: Linear Quadratic Methods*. Englewood Cliffs: Prentice-Hall.

Aoki, M. and Staley, R. M. (1970). On Input Signal Synthesis in Parameter Identification. *Automatica*, **6**, 431-440.

Arimoto, S. and Kimura, H. (1971). Optimum Input Test Signals for System Identification - An Information-Theoretical Approach. *Int.J.Systems Sci.*, **1**(3), 279-290.

Arulalan, G. R. and Deshpande, P. B. (1987). Simplified Model Predictive Control. *Ind.Eng.Chem.Res.*, **26**, 347-356.

Bailey, C. M. and Nicholson, H. (1989). A New Structured Model for Plant Cell Culture. *Biotech.Bioeng.*, **34**, 1331-1336.

Bajpai, R. K. and Reuss, M. (1980). A Mechanistic Model for Penicillin Production. *J.Chem.Tech.Biotechnol.*, **30**, 332-344.

Banerjee, R. and Bhattacharyya, B. C. (1993). Evolutionary Operation (EVOP) to Optimize Three-Dimensional Biological Experiments. *Biotech.Bioeng.*, **41**(1), 67-71.

Bastin, G., Chen, L. and Chotteau, V. (1992). Can We Identify Biotechnological Processes ? In M. N. Karim and G. Stephanopoulos (Eds.), *Modelling and Control of Biotechnical Processes 1992*. (pp. 83-88). Colorado: Pergamon Press.

Bastin, G. and Dochain, D. (1986). On-line estimation of microbial specific growth rates. *Automatica*, **22**(6), 705-709.

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

Bastin, G., Dochain, D., Haest, M., Installe, M. and Opdenacker, P. (1982). Modelling and Adaptive Control of a Continuous Anaerobic Fermentation Process. In *IFAC Modelling and Control of Biotechnical Processes*, (pp. 299-306). Helsinki, Finland.

Bellgardt, K.-H., Hopf, N., Luttmann, R. and Deckwer, W.-D. (1989). A New Approach for Development of Structured Growth Models. In N. M. Fish (Eds.), *Modelling and Control of Biotechnological Process* (pp. 79-91). Elsevier Applied Science.

Bestle, D. and Eberhard, P. (1994). Automated Approach for Optimizing Dynamic Systems. In R. Bulirsch and D. Kraft (Eds.), *Computational Optimal Control.* (pp. 225-235). Basel: Birkhauser Verlag.

Betts, J. T. (1989). Sparse Jacobian Updates in the Collocation Method for Optimal Control Problems. *J.Guidance*, **13**(3), 409-415.

Betts, J. T. and Huffman, W. P. (1990). Application of Sparse Nonlinear Programming to Trajectory Optimizaiton. *J.Guidance*, **15**(1), 198-206.

Biegler, L. T. (1991). Optimization Approaches to Nonlinear Model Predictive Control. In Y. Arkun and W. H. Ray (Ed.), *Chemical Process Control CPCIV. Proceedings of the Fourth International Conference on Chemical Process Control.*, (pp. 543-571). Padre Island, Texas: CACHE, AIChE.

Biryukov, V. V. (1982). Computer Control and Optimization of Microbial Metabolite Production. In *IFAC Modelling and Control of Biotechnical Processes*, (pp. 135-144). Helsinki, Finland.

Bitmead, R. R., Gevers, M. and Wertz, V. (1990). *Adaptive Optimal Control: The Thinking Man's GPC*. Prentice Hall.

Bosnjak, M., Stroj, A., Curcic, M., Adamovic, V. and Johanides, V. (1985). Application of Scale-Down Experiments in the Study of Kinetics of Oxytetracycline Biosynthesis. *Biotech.Bioeng.*, **27**, 398-408.

Bosnjak, M., Topolovec, V. and Johanides, V. (1979). Growth Kinetics and Antibiotic Synthesis during the Repeated Fed-Batch Culture of *Streptomycetes*. *Biotech.Bioeng.Symp.*, **9**, 155-165.

Bosnjak, M., Topolovec, V., Stroj, A. and johanides, V. (1981). Kinetics of Substrate Utilization in Repeated Fed Batch Culture and Fitting Computer Simulation Data to Experimental Data of Antibiotic Biosynthesis. In M. Moo-Young (Eds.), *Advances in Biotechnology*. (pp. 303-308). Pergamon Press.

Bosnjak, M., Topolovec, V. and Vrana, M. (1978). Growth Kinetics of *Streptomyces erythreus* During Erythromycin Biosysthesis. *J.Appl.Chem.Biotechnol.*, **28**, 791-798.

Box, G. E. P., Hunter, W. G. and Hunter, J. S. (1978). *Statistics for Experimenters : An Introduction to Design, Data Analysis and Model Building*. John Wiley & Sons.

Breedijk, T., Edgar, T. F. and Trachtenberg, I. (1994). Parameter Estimation and Nonlinear Predictive Control for RTP. In *IFAC Advanced Control of Chemical Processes.*, 1 (pp. 83-88). Kyoto, Japan: Pergamon Press.

Bryson, A. E. and Ho, Y. C. (1975). *Applied Optimal Control*. Hemisphere.

Cazzador, L. (1988). On The Optimal Control of Fed-Batch Reactors with Substrate-Inhibited Kinetics. *Biotech.Bioeng.*, **31**, 670-674.

Chang, Y. K. and Lim, H. C. (1989). Experimental and Simulation Studies of Multivariable Adaptive Optimisation of continuous Bioreactors Using Bilevel Forgetting Factors. *Biotech.Bioeng.*, **34**, 577-591.

Chang, Y. K. and Lim, H. C. (1990). Fast Inferential Adaptive Optimization of a Continuous Yeast Culture Based on Carbon Dioxide Evolution Rate. *Biotech.Bioeng.*, **35**, 8-14.

Chang, Y. K., Pyun, Y. R. and Lim, H. C. (1988). Adaptive Optimization of a Continuous Culture with the Use of a Bilevel Forgetting Factor. *Biotech.Bioeng.*, **31**, 944-957.

Charbonnier, S. and Cheruy, A. (1994). Estimation of State Variables and Kinetic Parameters in an Enzyme Production process by Using Minimal Modelling. In *The Third*

IEEE Conference on Control Applications., 3 (pp. 1923-1928). Strathclyde University, Glasgow

Chattaway, T. and Stephanopoulos, G. (1989). Adaptive Estimation of Bioreactors: Monitoring Plasmid Instability . *Chem.Eng.Sci.*, **44**(1), 41-48.

Chen, H.-C. (1994). Response-Surface Methodology for Optimising Citric Acid Fermentation by *Aspergillus foetidus*. *Process Biochem.*, **29**, 399-405.

Clarke, D. W., Mohtadi, C. and Tuffs, P. S. (1987a). Generalized Predictive Control - Part 1. The Basic Algorithm. *Automatica*, **23**(2), 137-148.

Clarke, D. W., Mohtadi, C. and Tuffs, P. S. (1987b). Generalized Predictive Control - Part 2. Extensions and Interpretations. *Automatica*, **23**(2), 149-160.

Cliffe, K. (1988). Bioreactors. In A. Scragg (Eds.), *Biotechnology for Engineering.* (pp. 177-301). Ellis Horwood Limited.

Cobelli, C. and Thomaseth, K. (1985). Optimal Input Design for Identification of Compartmental Models. Theory and Application to a Model of Glucose Kinetics. *Math.Biosci.*, **77**, 267-286.

Cooney, C. L., Wang, H. Y. and Wang, D. I. C. (1977). Computer-Aided Material Balancing for Prediction of Fermentation Parameters. *Biotech.Bioeng.*, **19**, 55-67.

Cooney, M. J. and McDonald, K. A. (1995). Optimal Dynamic Experiments for Bioreactor Model Discrimination. *Appl.Micro.Biotech.*, **43**, 826-837.

Cornet, J. F., Dussap, C. G., Cluzel, P. and Dubertret, G. (1992a). A Structured Model for Simulation of Cultures of the Cyanobacterium *Spirulina platensis* in Photobioreactors: II. Identification of Kinetic Parameters under Light and Mineral Limitations. *Biotech.Bioeng.*, **40**(7), 826-834.

Cornet, J. F., Dussap, C. G. and Dubertret, G. (1992b). A Structured Model for Simulation of Cultures of the Cyanobacterium *Spirulina platensis* in Photobioreactors: I. Coupling Between Light and Growth Kinetics. *Biotech.Bioeng.*, **40**(7), 817-825.

Crueger, W. and Crueger, A. (1989). *Biotechnology: A Textbook of Industrial Microbiology*. Sunderland, MA.: Sinauer Associates, Inc.

Cuthrell, J. E. and Biegler, L. T. (1987). On the Optimization of Differential-Algebraic Process Systems. *AIChE J.*, **33**(8), 1257-1270.

Cutler, C. R. and Ramaker, B. L. (1979). Dynamic Matrix Control - A Computer Control Algorithm. In *AIChE 86th National Meeting*, (paper 51b).

Dahhou, B., Chamilothoris, G. and Roux, G. (1991a). Adaptive Predictive Control of a Continuous Fermentation Process. *Int.J.Adapt.Cont.Sig.Proc.*, **5**, 351-362.

Dahhou, B., Roux, G., Queinnec, I. and Pourciel, J. B. (1991b). Adaptive Pole Placement Control of a Continuous Fermentation Process. *Int.J.Systems.Sci.*, **22**(12), 2625-2638.

Dahhou, B., Lakrori, M., Queinnec, I., Ferret, E. and Cheruy, A. (1992a). Control of a Continuous Fermentation Process. *J.Proc.Cont.*, **2**(2), 103-111.

Dahhou, B., Roux, G. and Chamilothoris, G. (1992b). Modelling and Adaptive Predictive Control of a Continuous Fermentation Process. *Appl.Math.Modelling*, **16**, 545-552.

Darouach, M. and Boutayeb, M. (1992). Recursive State and Parameter Estimation of SISO Singular Systems. *IEE.Proc.-D*, **139**(2), 204-206.

Dekkers, R. M. (1982). State Estimation of a Fed-Batch Baker's Yeast Fermentation. In *IFAC Modelling and Control of Biotechnical Processes*, (pp. 201-211). Helsinki, Finland.

Delgado, J. and Liao, J. C. (1992). Determination of Flux Control Coefficients from Transient Metabolite Concentrations. *Biochem.J.*, **282**, 919-927.

Dochain, D. (1990). Multivariable Adaptive Control of Nonlinear Completely Mixed Bioreactors. In *1990 American Control Conference*, (pp. 2661-2666). San Diego, California.

Dochain, D. and Bastin, G. (1985). Stable Adaptive Algorithms for Estimation and Control of Fermentation Processes. In A. Johnson (Ed.), *IFAC Modelling and Control of Biotechnological Processes*, (pp. 37-42). Noordwijkerhout.

Dochain, D. and Bastin, G. (1990). Adaptive Control of Fedbatch Bioreactors. *Chem.Eng.Comm.*, **87**, 67-85.

Dochain, D., Byul, E. D. and Bastin, G. (1989). Experimental Validation of a Methodology for online State Estimation in Bioreactors. In N. M. Fish (Eds.), *Modelling and Control of Biotechnological Process* (pp. 187-194). Elsevies Applied Science.

Eaton, J. W. and Rawlings, J. B. (1990). Feedback Control of Chemical Processes Using On-Line Optimization Techniques. *Comp.Chem.Eng.*, **14**(4/5), 469-479.

Eaton, J. W. and Rawlings, J. B. (1992). Model-Predictive Control of Chemical Processes. *Chem.Eng.Sci.*, **47**(4), 705-720.

Economou, C. G. and Morari, M. (1986a). Internal Model Control 5. Extension to Nonlinear Systems. *Ind.Eng.Chem.Process.Des.Dev.*, **25**, 403-411.

Economou, C. G. and Morari, M. (1986b). Internal Model Control 6. Multiloop Design. *Ind.Eng.Chem.Process.Des.Dev.*, **25**, 411-419.

Engrand, J. C. (1980). Applications of Multivariable Control in a Refinery and Implementation of a Dedicated Minicomputer. In *Joint Automatic Control Conference.*, (paper FA9-D). San Francisco.

Esener, A. A., Veerman, T., Roels, J. A. and Kossen, N. W. F. (1982). Modelling of Bacterial Growth ; Formulation and Evaluation of a Structured Model. *Biotech.Bioeng.*, **24**, 1749-1764.

Fan, Y., Sarkar, S. and Lasdon, L. (1988). Experiments with Successive Quadratic Programming Algorithms. *J.Opt.The.Appl.*, **56**(3), 359-383.

Filippi, C., Bordet, J., Villermaux, J. and Marchal, S. (1989). Batch Reactor Optimization by Use of Tendency Models. *Computer Chem.Eng.*, **13**(1/2), 35-47.

Filippi, C., Greffe, J. L., Bordet, J., Villermaux, J., Barnay, J. L., Bonte, P. and Georgakis, C. (1986). Tendency Modeling of Semibatch Reactors for Optimization and Control. *Chem.Eng.Sci.*, **41**(4), 913-920.

Flaus, J. M., Cheruy, A., Engasser, J. M., Poch, M. and Sola, C. (1991). Estimation of the State and Parameters in Bioprocesses from Indirect Measurements. In *ECC91 European Control Conferrence*, 2 (pp. 1643-1647). Grenoble, France.

Fredrickson, A. G. (1976). Formulation of Structured Growth Models. *Biotech.Bioeng.*, **18**, 1481-1486.

Froisy, J. B. and Richalet, J. (1986). Industrial Applications of IDCOM. In M. Morari (Ed.), *Proceedings of Chemical Process Control CPCIII.*, (pp. 233-243).

Garcia, C. E. and Morari, M. (1982). Internal Model Control 1. A Unifying Review and Some New Results. *Ind.Eng.Chem.Process.Des.Dev*, **21**, 308-323.

Garcia, C. E. and Morari, M. (1985a). Internal Model Control 2. Design Procedure for Multivariable Systems. *Ind.Eng.Chem.Process Des.Dev.*, **24**, 472-484.

Garcia, C. E. and Morari, M. (1985b). Internal Model Control 3. Multivariable Control Law Computation and Tuning Guidelines. *Ind.Eng.Chem.Process Des.Dev.*, **24**(2), 484-494.

Garcia, C. E. and Morshedi, A. M. (1986). Quadratic Programming Solution of Dynamic Matrix Control (QDMC). *Chem.Eng.Commun.*, **46**, 73-87.

Gattu, G. and Zafiriou, E. (1992). Nonlinear Quadratic Dynamic Matrix Control with State Estimation. *Ind.Eng.Chem.Res.*, **31**, 1096-1104.

Geraats, S. G. M., Hooijmans, C. M., Niel, E. W. J., Robertson, L. A., Heijnen, J. J., Luyben, K. C. A. M. and Kuenen, J. G. (1990). The Use of a Metabolically Structured Model in the Study of Growth, Nitrification, and Denitrification by *Thiosphaera pantotropha*. *Biotech.Bioeng.*, **36**, 921-930.

Ghoul, M., Pons, M.-N., Engasser, J.-M. and Bordet, J. (1985). Extended Kalman Filtering Technique for The On-Line Control of *Candida utilis* Production. In A. Johnson (Ed.), *IFAC Modelling and Control of Biotechnological Processes*, (pp. 185-189). Noordwijkerhout.

Giladi, R. and Sideman, S. (1989). Optimal Input Design: Case Study of the Metabolic Process. *Math.Biosci.*, **97**, 179-197.

Gill, P. E., Murray, W. and Saunders, M. A. (1994). Large-Scale SQP Methods and their Application in Trajectory Optimization. In R. Bulirsch and D. Kraft (Eds.), *Computational Optimal Control.* (pp. 29-42). Basel: Birkhauser Verlag.

Godfrey, K. (Ed.). (1993). *Perturbation Signals for System Identification*. New York: Prentice Hall.

Goh, C. J. and Teo, K. L. (1988). Control Parametrization: A Unified Approach to Optimal Control Problems with General Constraints. *Automatica*, **24**(1), 3-18.

Goodwin, G. C. (1969). Input Synthesis for Minimum Covariance State and Parameter Estimation. *Elec.Let.*, **5**(21), 539-540.

Goodwin, G. C. (1971). Optimal Input Signals for Nonlinear-System Identification. *Proc.IEE.*, **118**(7), 922-926.

Goodwin, G. C. and Payne, R. L. (1973). Design and Characterisation of Optimal Test Signals for Linear Single Input-Single Output Parameter Estimation. In *3rd IFAC Symp. on Identification and System Parameter Estimation*, (pp. 1005-1010).

Goodwin, G. C. and Payne, R. L. (1977). *Dynamic System Identification : Experiment Design and Data Analysis*. Academic Press.

Goodwin, G. C., Zarrop, M. B. and Payne, R. L. (1974). Coupled Design of Test Signals, Sampling Intervals, and Filters for System Identification. *IEEE Trans.Auto.Control*, **AC-19**(6), 748-752.

Green, M. and Limebeer, D. (1994). *Robust Control Systems*. Prentice Hall.

Grosz, R., Stephanopoulos, G. and San, K.-Y. (1984). Studies on On-line Bioreactor Identification 3. Sensitivity Problems with Respiration and Heat Evolution Measurements. *Biotech.Bioeng.*, **26**, 1198-1208.

Gulik, W. M., Hoopen, H. J. G. and Heijnen, J. J. (1993). A Structured Model Describing Carbon and Phosphate Limited Growth of *Catharanthus roseus* Plant Cell Suspensions in Batch and Chemostat Culture. *Biotech.Bioeng.*, **41**(8), 771-780.

Guthke, R. and Knorre, W. A. (1981). Optimal Substrate Profile for Antibiotic Fermentation. *Biotech.Bioeng.*, **23**, 2771-2777.

Hagander, P. and Holst, O. (1992). Substrate Control in Fedbatch Cultivations Using a Model-Based Modification of a PI-Regulator. In M. N. Karim and G. Stephanopoulos

(Eds.), *Modelling and Control of Biotechnical Processes 1992.* (pp. 77-82). Colorado: Pergamon Press.

Hamer, J. W. and Richenberg, C. B. (1988). On-Line Optimizing Control of a Packed-Bed Immobilized-Cell Reactor. *AIChE.J.*, **34**(4), 626-632.

Harder, A. and Roels, J. A. (1982). Application of Simple Structured Models in Bioengineering. *Adv.Biochem.Eng.*, **21**, 55-107.

Hargraves, C. R. and Paris, S. W. (1987). Direct Trajectory Optimization Using Nonlinear Programming and Collocation. *J.Guidance*, **10**(4), 338-342.

Hass, V. C. and Munack, A. (1990). Experimental Design of Fermentations for Model Identification. In *1990 American Control Conference*, 2 (pp. 1528-1533). San Diego, California.

Heijden, R. T. J. M. V. D., Hellinga, C., Luyben, K. C. A. M. and Honderd, G. (1989). State Estimators (Observers) for the On-line Estimation of non-measurable Process variables. *TIBTECH*, **7**, 205-209.

Hilaly, A. K., Karim, M. N. and Guyre, D. (1994). Optimization of an Industrial Microalgae Fermentation. *Biotech.Bioeng.*, **43**(4), 314-320.

Hilaly, A. K., Karim, M. N. and Linden, J. C. (1992). Real Time Application of Extended Kalman Filtering in Estimation and Optimization of a Recombinant *Escherichia coli* Fermentation. In M. N. Karim and G. Stephanopoulos (Eds.), *Modelling and Control of Biotechnical Processes 1992.* (pp. 371-373). Colorado: Pergamon Press.

Hilaly, A. K., Karim, M. N. and Linden, J. C. (1993). Studies on Real Time Optimization of a Fed-Batch Recombinant *Escherichia coli* Fermentation. In *12th World Congress International Federation of Automatic Control.*, 9 (pp. 121-124). Sydney, Australia.

Hitzmann, B., Schongarth, K., Lohn, A. and Weigel, B. (1995). Projective Reference Evaluation - The Reliable Evaluation Technique for Flow Injection Analysis Signals. In A. Munack and K. Schugert (Ed.), *6th International Conference on Computer Application in Biotechnology.*, (pp. 17-23). Garmisch-Partenkirchen, Germany: Elsevier.

Hong, J. (1986). Optimal Substrate Feeding Policy for a Fed Batch Fermentation with Substrate and Product Inhibition Kinetics. *Biotech.Bioeng.*, **28**, 1421-1431.

Hooker, B. S. and Lee, J. M. (1992). Application of a New Structured Model to Tobacco Cell Cultures. *Biotech.Bioeng.*, **39**(7), 765-774.

Jalel, N. A., Shui, F., Tang, R. and Leigh, J. R. (1992). Supervisory System for Modelling and Estimation of a Fermentation Process. In *6th IMA Conference on Control: Modelling, Computation, Information.*,. Manchester.

Jerome, N. F. and Ray, W. H. (1992). Model-Predictive Control of Linear Multivariable Systems Having Time Delays and Right-Half-Plane Zeros. *Chem.Eng.Sci.*, **47**(4), 763-785.

Johnson, A. (1987). The Control of Fed-Batch Fermentation Processes - A Survey. *Automatica*, **23**(6), 691-705.

Johnson, A. (1992). Optimization of Fed-Batch Biotechnical Processes. In M. N. Karim and G. Stephanopoulos (Eds.), *Modelling and Control of Biotechnical Processes 1992.* (pp. 243-246). Colorado: Pergamon Press.

Johnson, A. (1993). The Theory of LQ Optimization of Fermentation. In *12th World Congress International Federation of Automatic Control.*, 9 (pp. 115-119). Sydney, Australia.

Johnson, D. B. and Berthouex, P. M. (1975a). Efficient Biokinetic Experimental Designs. *Biotech.Bioeng.*, **17**, 557-570.

Johnson, D. B. and Berthouex, P. M. (1975b). Using Multiresponse Data to Estimate Biokinetic parameters. *Biotech.Bioeng.*, **17**, 571-583.

Kell, D. B., Dam, K. V. and Westerhoff, H. V. (1989). Control Analysis of Microbial Growth and Productivity. In S. Baunberg, I. Hunter and M. Rhodes (Eds.), *Microbial Products: New Approaches.*, (pp 61-96). Cambridge: Cambridge University Press.

Keulers, M., Ariaans, L., Giuseppin, M. and Soeterboek, R. (1993). Specific Growth Rate Control in Fed-Batch Bakers' Yeast Fermentation. In *12th World Congress International Federation of Automatic Control.*, 4 (pp. 441-444). Sydney, Australia.

Keulers, M., Ariaans, L., Soeterboek, R. and Giuseppin, M. (1994). Facilitating Control of Fed-Batch Fermentation Processes by Monitoring the Growth Rates of *Saccharomyces cerevisiae*. In *The Third IEEE Conference on Control Applications.*, 3 (pp. 1935-1940). Strathclyde University, Glasgow.

Kirk, D. E. (1970). *Optimal Control Theory: An Introduction*. Englewood Cliffs, New Jersey: Prentice-Hall.

Kompala, D. S., Ramkrishna, D., Jansen, N. B. and Tsao, G. T. (1986). Investigation of Bacterial Growth on Mixed Substrates: Experimental Evaluation of Cybernetic Models. *Biotech.Bioeng.*, **28**, 1044-1055.

Korte, G., Rinas, U., Krache-Helm, H.-A. and Schugerl, K. (1991). Structured Model for Cell Growth and Enzyme Production by Recombinant Escherichia coli. *Appl.Microbiol.Biotech.*, **35**, 185-188.

Kwakernaak, H. and Sivan, R. (1972). *Linear Optimal Control Systems*. New York: Wiley-Interscience.

Kwon, W. H. and Byun, D. G. (1989). Receding Horizon Tracking Control as a Predictive Control and its Stability Properties. *Int.J.Control.*, **50**(5), 1807-1824.

Laughlin, D. L., Jordan, K. G. and Morari, M. (1986). Internal Model Control and Process Uncertainty: Mapping Uncertainty Regions for SISO Controller Design. *Int.J.Control*, **44**(3), 1675-1698.

Lebourgeois, F. (1980). IDCOM Applications and Experiences on a PVC Production Plant. In *Joint Automatic Control Conference.*, (pp. paper FA9-C). San Francisco.

Lee, J. H., Gelormino, M. S. and Morari, M. (1992). Model Predictive Control of Multi-Rate Sampled-Data Systems: A State-Space Approach. *Int.J.Control*, **55**(1), 153-191.

Lee, J. H. and Morari, M. (1992). Robust Inferential Control of Multi-Rate Sampled-Data Systems. *Chem.Eng.Sci.*, **47**(4), 865-885.

Lee, S. Y., Coronella, C. J., Bhadkamkar, A. S. and Seader, J. D. (1993). Modelling and Temperature Regulation of a Thermally Coupled Reactor System via Internal Model Control Strategy. *Ind.Eng.Chem.Res.*, **32**(12), 3029-3036.

Lee, W. and V.W. Weekman, J. (1976). Advanced Control Practice in the Chemical Process Industry: A View from Industry. *AIChE.J.*, **22**(1), 27-38.

Liao, J. C. and Delgado, J. (1992). Dynamic Metabolic Control Theory. In H. Pedersen, R. Mutharasan and D. DiBiasio (Eds.), *Biochemical Engineering VII.* (pp. 27-38).

Lim, H. C., Tayeb, Y. J., Modak, J. M. and Bonte, P. (1986). Computational Algorithms for Optimal Feed Rates for a class of Fed-Batch Fermentation: Numerical Results for Penicillin and cell Mass Production. *Biotech.Bioeng.*, **28**, 1408-1420.

Liu, Y.-C., Tsao, J.-H., Chang, W.-M. and Wu, W.-T. (1992). On-Line State Estimation for L-Lysine Fermentation. In M. N. Karim and G. Stephanopoulos (Eds.), *Modelling and Control of Biotechnical Processes 1992.* (pp. 279-282). Colorado: Pergamon Press.

Luedeking, R. and Piret, E. L. (1959). A Kinetic Study of the Lactic Acid Fermentation: Batch Process at Controlled pH. *J.Biochem.Micro.Tech.Eng.*, **1**(4), 393-412.

Luus, R. (1993a). Optimization of Fed-Batch Fermentors by Iterative Dynamic Programming. *Biotech.Bioeng.*, **41**, 599-602.

Luus, R. (1993b). Piecewise Linear Continuous Optimal Control by Iterative Dynamic Programming. *Ind.Eng.Chem.Res.*, **32**(5), 859-865.

Mayne, D. Q. and Michalska, H. (1990). Receding Horizon Control of Nonlinear Systems. *IEEE Trans.Auto.Control*, **35**(7), 814-824.

McDonald, K. A. and McAvoy, T. J. (1987). Application of Dynamic Matrix Control to Moderate and High Purity Distillation Towers. *Ind.Eng.Chem.Res.*, **26**(5), 1011-1018.

Mehlhorn, R., Dinkelmann, M. and Sachs, G. (1994). Application of Automatic Differentiation to Optimal Control Problems. In R. Bulirsch and D. Kraft (Eds.), *Computational Optimal Control.* (pp. 255-267). Basel: Birkhauser Verlag.

Mehra, R. K. (1974). Optimal Input Signals for Parameter Estimation in Dynamic Systems - Survey and New Results. *IEEE Trans.Auto.Control*, **AC-19**(6), 753-768.

Mehra, R. K. and Eterno, J. S. (1980). Model Algorithmic Control for Electric Power Plant. In *IEEE Conference on Decision and Control.*, (pp. 280-285). Alber-querque.

Mehra, R. K., Rouhani, R. and Eterno, J. (1982). Model Algorithmic Control (MAC); Review and Recent Developments. In *Engineering Foundation Conference on Chemical Process Control 2.*, (pp. 287-309). Sea Island, Georgia.

Melin, C., Laanait, A., Delarue, F. and Cordonnier, M. (1982). Direct Digital Control and Adaptive Control Algorithms for a Pilot Scale Fermentor: Some Applications. In *IFAC Modelling and Control of Biotechnical Processes*, (pp. 283-289). Helsinki, Finland.

Miele, A. and Wang, T. (1986). Primary-Dual Properties of Sequential Gradient-Restoration Algorithms for Optimal Control Problems 2. General Problem. *J.Math.Anal.Appl.*, **119**, 21-54.

Mirzai, A. R., Dixon, K., Hinge, R. D. and Leigh, J. R. (1991). Approaches to the Modelling of Biochemical Processes. In *Intermational Conference on Control'91*, 2 (pp. 844-849).

Mirzai, A. R., Leigh, J. R., Hou, Y. C. and Keshavarz, T. (1990). Application of Kalman Filter Estimators to a Fed-Batch Fermentation Process. In E. Arikan (Eds.), *Communication, Control and Signal Processing* (pp. 1718-1724). Elsevier Science Publishers B.V.

Modak, J. M. and Lim, H. C. (1987). Feedback Optimization of Fed-Batch Fermentation. *Biotech.Bioeng.*, **30**, 528-540.

Modak, J. M. and Lim, H. C. (1989). Simple Nonsingular Control Approach to Fed-Batch Fermentation Optimization. *Biotech.Bioeng.*, **33**, 11-15.

Modak, J. M., Lim, H. C. and Tayeb, Y. J. (1986). General Characteristics of Optimal Feed Rate Profiles for various Fed-Batch Fermentation Processes. *Biotech.Bioeng.*, **28**, 1396-1407.

Monod, J. (1949). The Growth of Bacterial Cultures. *Ann.Rev.Microb.*, **3**, 371-394.

Montague, G. A., Morris, A. J. and Tham, M. T. (1989). Adaptive Inferential Estimation and Its Application to Biomass Control. In N. M. Fish (Eds.), *Modelling and Control of Biotechnological Process* (pp. 179-186). Elsevier Applied Science.

Montague, G. A., Morris, A. J., Wright, A. R., Aynsley, M. and Ward, A. (1985). Parameter Adaptive Control of The Fed-Batch Penicillin Fermentation. In A. Johnson (Ed.), *IFAC Modelling and Control of Biotechnological Processes*, (pp. 69-74). Noordwijkerhout.

Montgomery, P. A., Williams, D. and Swanick, B. H. (1985). Control of Fermentation Process by an On-line Adaptive Technique. In *IFAC Modelling and Control of Biotechnological Process*, (pp. 111-119).

Mosca, E. and Zhang, J. (1992). Stable Redesign of Predictive Control. *Automatica*, **28**(6), 1229-1233.

Munack, A. (1989). Optimal Feeding Strategy For Identification of Monod-Type Models by Fed-Batch Experiments. In N. M. Fish (Eds.), *Modelling and Control of Biotechnological Process.* (pp. 195-204). Elsevier Applied Science.

Munack, A. and Posten, C. (1989). Design of Optimal Dynamical Experiments for Parameter Estimation. In *1989 American Control Conference*, (pp. 2010-2016). Pittsburgh, PA.

Muske, K. R. and Rawlings, J. B. (1993a). Linear Model Predictive Control of Unstable Processes. *J.Proc.Cont.*, **3**(2), 85-96.

Muske, K. R. and Rawlings, J. B. (1993b). Model Predictive Control with Linear Models. *AIChE.J.*, **39**(2), 262-287.

Nahlik, J. and Burianec, Z. (1988). On-Line Parameter and State Estimation of Continuous Cultivation by Extended Kalman Filter. *Appl.Microbiol.Biotechnol.*, **28**, 128-134.

Nestaas, E. and Wang, D. I. C. (1983). Computer Control of the Penicillin Fermentation Using the Filtration Probe in Conjunction with a Structured Process Model. *Biotech.Bioeng.*, **25**, 781-796.

Nicolai, B. M., Impe, J. F. V., Vanrolleghem, P. A. and Vandewalle, J. (1991). A Modified Unstructured Mathemathical Model for the Pencillin G Fed-Batch Fermentation. *Biotech.Lett.*, **13**(7), 489-494.

Nielsen, J. (1993). A Simple Morphologically Structured Model Describing the Growth of Filamentous Microorganisms. *Biotech.Bioeng.*, **41**(7), 715-727.

Nielsen, J. and Jorgensen, H. S. (1995). A Kinetic Model for the Penicillin Biosynthetic Pathway in *Pencillium chrysogenum.* In A. Munack and K. Schugert (Ed.), *6th International Conference on Computer Application in Biotechnology.*, (pp. 136-141). Garmisch-Partenkirchen, Germany: Elsevier.

Nielsen, J., Nikolajsen, K. and Villadsen, J. (1991a). Structured Modelling of a Microbial System: 1. A Theoretical Study of Lactic Acid Fermentation. *Biotech.Bioeng.*, **38**, 1-10.

Nielsen, J., Nikolajsen, K. and Villadsen, J. (1991b). Structured Modelling of a Microbial System: 2. Experimental Verification of A Structured Lactic Acid Fermentation Model. *Biotech.Bioeng.*, **38**, 11-23.

Nielsen, J., Nikolajsen, K. and Villadsen, J. (1991c). Structured Modelling of a Microbial System: 3. Growth on Mixed Substrates. *Biotech.Bioeng.*, **38**, 24-29.

Nielsen, J., Pedersen, A. G., Strudsholm, K. and Villadsen, J. (1991d). Modeling Fermentations with Recombinant Microorganisms: Formulation of a Structured Model. *Biotech.Bioeng.*, **37**, 802-808.

Noton, M. (1972). *Modern Control Engineering*. Pergamon Press.

O'Neil, D. G. and Lyberatos, G. (1990). Dynamic Model Development for a Continuous Culture of *Saccharomyces cerevisiae*. *Biotech.Bioeng.*, **36**, 437-445.

Ogunnaike, B. A. and Adewale, K. E. P. (1986). Dynamic Matrix Control for Process Systems with Time Varying Parameters. *Chem.Eng.Commun.*, **47**, 295-314.

Ohno, H., Nakanishi, E. and Takamatsu, T. (1976). Optimal control of a semibatch fermentation. *Biotech.Bioeng.*, **18**, 847-864.

Ohno, H., Nakanishi, E. and Takamatsu, T. (1978). Optimum Operating Mode for a Class of Fermentation. *Biotech.Bioeng.*, **20**, 625-636.

Ozgulsen, F., Kendra, S. J. and Cinar, A. (1993). Nonlinear Predictive Control of Periodically Forced Chemical Reactors. *AIChE.J.*, **39**(4), 589-598.

Palsson, B. O. and Joshi, A. (1987). On the dynamic order of structured *Escherichia coli* growth models. *Biotech.Bioeng.*, **29**, 789-792.

Park, S. and Ramirez, W. F. (1988). Optimal Production of Secreted Protein in Fed-Batch Reactors. *AIChE.J.*, **34**(9), 1550-1558.

Park, S. H., Hong, K. T., Lee, J. H. and Bae, J. C. (1983). On-Line estimation of Cell Growth for Glutamic Acid Fermentation System. *Eur.J.Appl.Microbiol.Biotechnol.*, **17**, 168-172.

Parulekar, S. J. and Lim, H. C. (1985). Modeling, optimization and control of semi-batch bioreactors. *Adv.Biochem.Eng.*, **32**, 207-258.

Pomerleau, Y. and Viel, G. (1992). Industrial Application of Adaptive Nonlinear Control for Bakers' Yeast Production. In M. N. Karim and G. Stephanopoulos (Eds.), *Modelling and Control of Biotechnical Processes 1992.* (pp. 315-318). Colorado: Pergamon Press.

Porter, N. and Fox, F. M. (1993). Diversity of Microbial Products - Discovery and Application. *Pestic.Sci.*, **39**, 161-168.

Quinlan, A.V. (1986). Kinetics of Secondary Metabolite Synthesis in Batch Culture When Two Different Substrates Limit Cell Growth and Metabolite Production: Xanthan Synthesis by *Xanthomonas campestris*., in *Biochemical Engineering IV.*, H.C. Lim and K. Venkatasubramanian, Editor. pp. 259-269.

Ramirez, W. F. (1987). Optimal State and Parameter Identification. An Application to Batch Fermentation. *Chem.Eng.Sci.*, **42**(11), 2749-2756.

Ramirez, W. F. (1989). *Computational Methods for Process Simulation.* Boston London: Butterworths.

Ramirez, W. F. (1994). *Process Control and Identification.* London: Academic Press, INC.

Reinikainen, P., Olkku, J., Markkanen, P. and Holmberg, A. (1985). Determination of pH and Temperature Profiles for the Growth of *Escherichia coli.* In A. Johnson (Ed.), *IFAC Modelling and Control of Biotechnological Processes,* (pp. 219-224). Noordwijkerhout.

Renfro, J. G., Morshedi, A. M. and Asbjornsen, O. A. (1987). Simultaneous Optimization and Solution of Systems Described by Differential/Algebraic Equations. *Comp.Chem.Eng.*, **11**(5), 503-517.

Richalet, J., Rault, A., Testud, L. and Papon, J. (1978). Model Predictive Heuristic Control: Applications to Industrial Processes. *Automatica*, **14**, 413-428.

Rivera, D. E., Morari, M. and Skogestad, S. (1986). Internal Model Control 4. PID Controller Design. *Ind.Eng.Chem.Process.Des.Dev.*, **25**, 252-265.

Rizzi, M., Baltes, M., Mailinger, W., Theobald, U. and Reuss, M. (1995). Modelling of Short Term Crabtree-Effect in Baker's Yeast. In A. Munack and K. Schugert (Ed.), *6th International Conference on Computer Application in Biotechnology.*, (pp. 124-129). Garmisch-Partenkirchen, Germany: Elsevier.

Roels, J. A. (1983). *Energetics and Kinetics in Biotechnology.* Amsterdam: Elsevier Biomedical Press.

Rolf, M. J. and Lim, H. C. (1984). Adaptive On-Line Optimisation for Continuous Bioreactors. *Chem.Eng.Commun.*, **29**, 229-255.

Rolf, M. J. and Lim, H. C. (1985). Experimental Adaptive On-Line Optimization of Cellular Prodcutivity of a Continuous Bakers' Yeast Culture. *Biotech.Bioeng.*, **27**, 1236-1245.

Rose, A. H. (1979). Production and Industrial Importance of Secondary Products of Metabolism. In A.H. Rose (Ed.), *Economic Microbiology Vol. 3, Secondary Products of Metabolism.* (pp 1-33). Academic Press, London

Rosen, J. B. (1960). The Gradient Projection Method for Nonlinear Programming. Part 1. Linear Constraints. *J.Soc.Indust.Appl.Math.*, 8(1), 181-217.

Rosen, J. B. (1966). Iterative Solution of Nonlinear Optimal Control Problems. *J.SIAM Control Series A*, 223-244.

Rouhani, R. and Mehra, R. K. (1982). Model Algorithmic Control (MAC); Basic Theoretical Properties. *Automatica*, **18**(4), 401-414.

Roux, G., Dahhou, B., Najim, K. and Queinnec, I. (1992). Adaptive Linear Quadratic Gaussian (LQG) Control of a Bioreactor. *J.Chem.Tech.Biotechnol.*, **53**, 133-141.

Ruppen, D., Benthack, C. and Bonvin, D. (1994). Efficient Computation of Batch Reactor Control Profiles under Parametric Uncertainty. In *IFAC Advanced Control of Chemical Processes.*, (pp. 77-82). Kyoto, Japan: Pergamon Press.

Samaan, M., Dahhou, T., Queinnec, I. and Msaad, M. (1990). Experimental Results in Adaptive Control of Biotechnological Processes. In *1990 American Control Conference*, (pp. 2679-2683). San Diego, California.

San, K.-Y. and Stephanopoulos, G. (1984a). Studies on On-line Bioreactor Identification 2. Numerical and Experimental Results. *Biotech.Bioeng.*, **26**, 1189-1197.

San, K.-Y. and Stephanopoulos, G. (1984b). Studies on On-line Bioreactor Identification 4. Utilization of pH Measurements for Product Estimation. *Biotech.Bioeng.*, **26**, 1209-1218.

Saucedo, V. M., Valentinotti, S. C., Karim, M. N. and Collins, H. W. (1995). Feedback Control of a Recombinant Fed-Batch Fermentation Using On-Line HPLC Measurements. In A. Munack and K. Schugert (Ed.), *6th International Conference on Computer Application in Biotechnology.*, (pp. 24-28). Garmisch-Partenkirchen, Germany: Elsevier.

Scali, C., Semino, D. and Morari, M. (1992). Comparison of Internal Model Control and Linear Quadratic Optimal Control for SISO Systems. *Ind.Eng.Chem.Res.*, **31**(8), 1920-1927.

Schopf, R. and Deuflhard, P. (1994). OCCAL A Mixed Symbolic-Numeric Optimal Control CALculator. In R. Bulirsch and D. Kraft (Eds.), *Computational Optimal Control.* (pp. 269-278). Basel: Birkhauser Verlag.

Scragg, A. (1988). Biotechnology. In A. Scragg (Eds.), *Biotechnology for Engineering.* (pp. 19-24). Ellis Horwood Limited.

Semino, D., Scali, C. and Brambilla, A. (1993). Design of Double Filter IMC Controller for Ill-Conditioned Distillation columns. *Computers Chem.Eng.*, **17**(10), 1041-1046.

Semones, G. B. and Lim, H. C. (1989). Experimental Multivariable Adaptive Optimisation of the Steady-State Cellular Productivity of a Continuous Baker's Yeast Culture. *Biotech.Bioeng.*, **33**, 16-25.

Shi, Z., Shimizu, K., Iijima, S., Morisue, T. and Kobayashi, T. (1990). Adaptive On-Line Optimizing Control for Lactic Acid Fermentation. *J.Ferment.Bioeng.*, **70**(6), 415-419.

Shi, Z., Shimizu, K., Watanabe, N. and Kobayashi, T. (1989). Adaptive On-Line Optimization Control of Bioreactor Systems. *Biotech.Bioeng.*, **33**, 999-1009.

Shimizu, H., Araki, K., Shioya, S. and Suga, K.-I. (1991). Optimal Production of Glutathione by Controlling the Specific Growth Rate of Yeast in Fed-Batch Culture. *Biotech.Bioeng*, **38**, 196-205.

Shimizu, H., Miura, K., Takiguchi, N., Shioya, S. and Suga, K.-I. (1995). On-Line Physiological State Recognition and Parameter Estimation in the Metabolic Reaction Model Using Error Vectors. In A. Munack and K. Schugert (Ed.), *6th International Conference on Computer Application in Biotechnology.*, (pp. 118-123). Garmisch-Partenkirchen, Germany: Elsevier.

Shimizu, H., Shioya, S., Suga, K.-I. and Takamatsu, T. (1989a). Profile Control of the Specific Growth Rate in Fed-Batch Experiments. *Appl.Microbiol.Biotechnol.*, **30**, 276-282.

Shimizu, H., Takamatsu, T., Shioya, S. and Suga, K.-I. (1989b). An Algorithmic Approach to Construction the On-Line Estimation System for the Specific Growth Rate. *Biotech.Bioeng.*, **33**, 354-364.

Shioya, S., Shimizu, H., Ogata, M. and Takamatsu, T. (1985). Simulation and Experimental Studies of The Profile Control of the Specific Growth Rate in a Fed-Batch Culture. In *IFAC Modelling and Control of Biotechnological Processes*, (pp. 79-84). Noordwijkerhout, The Netherlands.

Sistu, P. B. and Bequette, B. W. (1991). Nonlinear Predictive Control of Uncertain Processes: Application to a CSTR. *AIChE.J.*, **37**(11), 1711-1723.

Stanbury, P. F. and Whitaker, A. (1993). *Principles of Fermentation Technology*. Oxford: Pergamon Press.

Staunton, J. (1978). *Primary Metabolism: A Mechanistic Approach.* Clarendon Press, Oxford.

Steinbach, M. C. (1994). A Structured Interior Point SQP Method for Nonlinear Optimal Control Problems. In R. Bulirsch and D. Kraft (Eds.), *Computational Optimal Control.* (pp. 213-222). Basel: Birkhauser Verlag.

Stephanopoulos, G. (1984). *Chemical Process Control: An Introduction to Theory and Practice*. Prentice Hall International, Inc.

Stephanopoulos, G. and San, K.-Y. (1981). State Estimation for Computer Control of Biochemical Reactors. In M. Moo-Young (Eds.), *Advances in Biotechnology.* (pp. 399-406). Pergamon Press.

Stephanopoulos, G. and San, K.-Y. (1984). Studies on On-line Bioreactor Identification 1. Theory. *Biotech.Bioeng.*, **26**, 1176-1188.

Takamatsu, T., Shioya, S. and Okada, Y. (1985). Profile Control Scheme in a Bakers' Yeast Fed-Batch Culture. *Biotech.Bioeng.*, **27**, 1675-1686.

Tang, R., Jalel, N. A., Mirzai, A. R. and Leigh, J. R. (1992). Identification and Modelling of Fermentation Process Using Matlab: Case Study. In *ICCAFT*,. Colorado, USA.

Teo, K. L., Goh, C. J. and Lim, C. C. (1989). A Computational Method for a Class of Dynamical Optimization Problems in which the Terminal Time is Conditionally Free. *IMA J.Math.Cont.Inform.*, **6**, 81-95.

Teo, K. L., Goh, C. J. and Wong, K. H. (1991). *A Unified Computational Approach to Optimal Control Problems*. Essex, UK: Longman Scientific and Technical.

Terwiesch, P. (1995). Cautious On-Line Correction of Batch Process Operation. *AIChE J.*, **41**(5), 1337-1340.

Terwiesch, P and Agarwal, M. (1995). Robust input polocies for batch reactors under parametric uncertainty. *Chem.Eng.commun.*, **131**, 33-52.

Terwiesch, P., Agarwal, M. and Rippin, D.W.T. (1994). Batch Unit Optimization with Imperfect Modelling: A Survey. *J.Proc.Cont.*, **4**(4), 238-258.

Thatipamala, R., Hill, G. A. and Rohani, S. (1993). On-Line State and Parameter Estimation and Adaptive Optimization of a Continuous Bioreactor (Ethanol Fermentation) Using State Equations. In *American Control Conference 1994*, 1 (pp. 905-909). San Francisco, California.

Tremblay, M. D., Perrier, M., Chavarie, C. and Archambault, J. (1990). Control of Fed-Batch Culture of Hybridoma Cells for Monoclonal Antibody Production. In *1990 American Control Conference*, (pp. 2150-2155). San Diego, California.

Tremblay, M. D., Perrier, M., Chavarie, C. and Archambault, J. (1992). Optimization of Fed-Batch Culture of Hybridoma Cells Using Dynamic Programming: Single and Multi Feed Cases. *Bioproc.Eng.*, **7**, 229-234.

Tremblay, M. D., Perrier, M., Chavarie, C. and Archambault, J. (1993). Fed-Batch Culture of Hybridoma Cells: Comparison of Optimal Control Approach and Closed Loop Strategies. *Bioproc.Eng.*, **9**, 13-21.

Tsao, J.-H., Chuang, H.-L. and Wu, W.-T. (1991). On-Line State Estimation and Control in Glutamic Acid Production. *Bioprocess.Eng.*, **7**, 35-39.

Van Impe, J. F. and Bastin, G. (1993). Optimal Adaptive Control of Fed-Batch Fermentation Processes with Multiple Substrates. In *Second IEEE Conference on Control Applications.*, 1 (pp. 469-474). Vancouver, B.C.

Van Impe, J. F., Bastin, G., Moor, B. D., Breusegem, V. V. and Vandewalle, J. (1992). Optimal Adaptive Control of Fed-Batch Fermentation Processes with Growth/Production Decoupling. In M. N. Karim and G. Stephanopoulos (Eds.), *Modelling and Control of Biotechnical Processes 1992.* (pp. 351-354). Colorado: Pergamon Press.

Van Impe, J. F., Nicolai, B. M., Vanrolleghem, P. A., Spriet, J. A., Moor, B. D. and Vandewalle, J. (1991). Optimal Control of the Penicillin G Fed-Batch Fermentation: An Analysis of a New Unstructured Model. In *ECC91 European Control Conferrence*, 2 (pp. 1624-1629). Grenoble, France.

Van Impe, J. F., Nicolai, B. M., Vanrolleghem, P. A., Spriet, J. A., Moor, B. D. and Vandewalle, J. (1992). Optimal Control of the Penicillin G Fed-Batch Fermentation: An Analysis of a Modified Unstructured Model. *Chem.Eng.Comm.*, **117**, 337-353.

Vanichsriratana, W., Keshavarz, T. and Leigh, J. R. (1993). Experimental Design for Parameter Estimation of Biochemical Processes. In *IChemE. 1993 Conference*, 1 (pp. 49-51). Birmingham: IChemE.

Vanichsriratana, W., Keshavarz, T. and Leigh, J. R. (1994). Modelling of Biochemical Processes - A Case of Complex Media Fermentation. In *IChemE. 1994 Conference*, 1 (pp. 132-134). University College, London: IChemE.

Vialas, C., Cheruy, A. and Gentil, S. (1985). An Experimental Approach to Improve the Monod Model Identification. In *IFAC Modelling and Control of Biotechnological Processes*, (pp. 175-179). Noordwijkerhout.

Vigie, P., Dohhou, B., Queinnec, I., Lakrori, M., Cheruy, A. and Pourciel, J. B. (1991). Control of Substrate Concentration in a Continuous bioprocess. *Bioprocess Engineering*, **6**, 259-263.

Wang, D. I. C., Cooney, C. L., Demain, A. L., Dunnill, P., Humphrey, A. E. and Lilly, M. D. (1979). *Fermentation and Enzyme Technology*. John Wiley & Sons.

Weigand, W. A., Lim, H. C., Creagan, C. C. and Mohler, R. D. (1979). Optimization of a Repeated Fed-Batch Reactor for Maximum Cell Productivity. *Biotech.Bioeng.Symp.*, **9**, 335-348.

Wiechert, W., Graaf, A. A. D. and Marx, A. (1995). In Vivo Stationary Flux Determination Using 13C NMR Isotope Labelling Experiments. In A. Munack and K. Schugert (Ed.), *6th International Conference on Computer Application in Biotechnology.*, (pp. 130-135). Garmisch-Partenkirchen, Germany: Elsevier.

Wiener, N. (1942). The Extrapolation, Interpolation and Smoothing of Stationary Time Series. NDRC Report, Cambridge, M.A.

Williams, D. and Montgomery, P. A. (1986). Multivariable Adaptive Control of Baker's Yeast Fermentation. *IEE Proceeding-D*, **133**(5), 247-253.

Williams, D., Yousefpour, P. and Swanick, B. H. (1984). Online Adaptive Control of a Fermentation Process. *IEE Proc.-D*, **131**(4), 117-124.

Williams, D., Yousefpour, P. and Wellington, E. M. H. (1986). On-Line Adaptive Control of a Fed-Batch Fermentation of *Saccharomyces cerevisiae*. *Biotech.Bioeng.*, **28**, 631-645.

Yamane, T. and Shimizu, S. (1984). Fed-Batch Techniques in Microbial Processes. *Adv.Biochem.Eng.*, **30**, 148-192.

Yeo, Y. K. and Williams, D. C. (1987). Bilinear Model Predictive Control. *Ind.Eng.Chem.Res.*, **26**, 2267-2274.

Yoo, Y. J., Marino-Galarraga, M., Hong, J. and Hatch, R. T. (1986). Experimental Design for Parameter Estimation from Batch Cultrue. *Biotech.Bioeng.*, **28**, 836-841.

Yoshida, F., Yamane, T. and Takamoto, K.-I. (1973). Fed-Batch Hydrocarbon Fermentation with Colloidal Emulsion Feed. *Biotech.Bioeng.*, **15**, 257-270.

Young, T. B. and Bungay, H. R. (1973). Dynamic Analysis of a Microbial Process: A Systems Engineering Approach. *Biotech.Bioeng.*, **15**, 377-393.

Yuan, J. Q. and Bellgardt, K. H. (1992). Structured Segregated Model for Optimal Profit Control of Baker's Yeast Production. In M. N. Karim and G. Stephanopoulos (Eds.), *Modelling and Control of Biotechnical Processes 1992.* (pp. 53-58). Colorado: Pergamon Press.

Zafiriou, E. and Morari, M. (1988). A General Controller Synthesis Methodology Based on the IMC Structure and the H_2-, H_∞-, and μ- Optimal Control Theories. *Comput.Chem.Eng.*, **12**(7), 757-765.

Zafiriou, E. and Morari, M. (1991). Internal Model Control: Robust Digital Controller Synthesis for Multivariable Open-Loop Stable or Unstable Processes. *Int.J.Control*, **54**(3), 665-704.

Zarrop, M. B. (1979). *Optimal Experiment Design for Dynamic System Identification.* Berlin: Springer-Verlag.

Zeng, F. Y., Dahhou, B., Goma, G. and Nihtila, M. T. (1992). Adaptive Estimation and Control of the Specific Growth Rate of a Nonlinear Fermentation Process via MRAC Method. In M. N. Karim and G. Stephanopoulos (Eds.), *Modelling and Control of Biotechnical Processes 1992.* (pp. 363-366). Colorado: Pergamon Press.

Zhang, B. S., Tang, R., Leigh, J. R., Dixon, K. D. and Hinge, R.D. (1996). Improved Quality and Productivity in Secondary Metabolite Fermentation through Estimation, Control and Scheduling. In *Proc. 13th IFAC World Congress*, San Francisco.

Appendix A Optimal Control Theory and Calculus of Variation.

Calculus of variation or Lagrange optimisation is a natural method for dynamical optimisation. In this appendix, we briefly give an introduction to this subject, which has been used in the thesis. Since the material presented here refers to a minimisation, it is therefore noted that maximisation of J(u) is equivalent to minimisation of -J(u). Where J(u) is a functional we want to minimise. The reader should consult the material in (Bryson and Ho, 1975; Kirk, 1970; Noton, 1972; Ramirez, 1994) for more detail on this subject.

In this appendix, we divide the material into three parts - Unconstrained control variable, Constrained control variable or Pontryagin's maximum principle and Necessary and sufficient conditions.

1. Unconstrained control variable

We want to minimise the following objective functional (J) from time t_0 to t_f ;

$$J(u) = \Phi\left[x(t_f)\right] + \int_{t_0}^{t_f} L\left[x(t), u(t), t\right] dt \qquad \text{(A-1)}$$

by using u(t), which is control inputs, subject to the constraint of the state equations

$$\dot{x} = \phi(x, u, t) \qquad \text{(A-2)}$$

The initial condition of the state equations (x(0)) are given. Φ, L and ϕ are assumed to be continuous with continuous first partial derivatives. For the application on fed-batch fermentation processes in this thesis, u is referred to substrate feed rate (F) or substrate concentration (S), Φ is a function of biomass (X) and/or product (P) at the final time and the state equations constraints (ϕ) are process models.

We include the constraints (A-2) into (A-1) and minimise this new objective functional ;

$$\hat{J}(u) = \Phi\left[x(t_f)\right] + \int_{t_0}^{t_f} \left\{ L(x,u,t) + \lambda^T \left[\phi(x,u,t) - \dot{x} \right] \right\} dt \qquad \text{(A-3)}$$

The Hamiltonian is then defined as:

$$H = L(x,u,t) + \lambda^T \phi(x,u,t) \qquad \text{(A-4)}$$

Where λ are called costates or dynamic Lagrange multipliers.

Equation (A-3) then becomes:

$$\hat{J}(u) = \Phi\left[x(t_f)\right] + \int_{t_0}^{t_f} \left(H - \lambda^T \dot{x} \right) dt$$

or

$$\hat{J}(u) = \left[\Phi(x) - \lambda^T x\right]_{t_f} + \left[\lambda^T x\right]_{t_0} + \int_{t_0}^{t_f} \left(H + \dot{\lambda}^T x \right) dt \qquad \text{(A-5)}$$

The first variation of (A-5) is:

$$\begin{aligned} \delta \hat{J}(u) = {} & \left[(\Phi_x - \lambda)^T \delta x \right]_{t_f} + \left[\lambda^T \delta x \right]_{t_0} + \left[\left(H + \frac{\partial \Phi}{\partial t} \right) \delta t \right]_{t_f} \\ & + \int_{t_0}^{t_f} \left(H_u^T \delta u + H_x^T \delta x + \dot{\lambda}^T \delta x \right) dt \end{aligned} \qquad \text{(A-6)}$$

In order to minimise the objective functional ($\hat{J}$), it is necessary that the first variation in Equation (A-6) equals zero. The following conditions ((A-7) to (A-10)) then constitute necessary conditions for this optimisation problem:

$$\frac{\partial H}{\partial u} = 0 \qquad \text{(A-7)}$$

$$\dot{\lambda}^T = -\frac{\partial H^T}{\partial x} \tag{A-8}$$

$$\left[(\Phi_x - \lambda)^T \delta x\right]_{t_f} + \left[\left(H + \frac{\partial \Phi}{\partial t}\right)\delta t\right]_{t_f} = 0 \tag{A-9}$$

Note that $[\lambda^T \delta x]_{t0}$ equal zero since the initial states have already been given. Since the state equation constraints must also be satisfied:

$$\dot{x} = \frac{\partial H}{\partial \lambda} = \phi(x, u, t) \tag{A-10}$$

Therefore, minimising $\hat{J}$ in (A-3) is equivalent to minimise J in (A-1).

Equation (A-9) is also known as the transversality condition or final condition. For a system with free final state and free final time, the transversality condition gives the following results:

$$\lambda(t_f) = \frac{\partial \Phi}{\partial x}(t_f) \tag{A-11}$$

$$H(t_f) = \frac{\partial \Phi}{\partial t}(t_f) = 0 \tag{A-12}$$

It can also be shown form the time derivative of the Hamiltonian that:

$$\frac{dH}{dt} = \left(\frac{\partial H}{\partial u}\right)^T \dot{u} + \left(\frac{\partial H}{\partial x}\right)^T \dot{x} + \left(\frac{\partial H}{\partial \lambda}\right)^T \dot{\lambda}$$

$$= \left(\frac{\partial H}{\partial u}\right)^T \dot{u} + \left(-\dot{\lambda}^T \dot{x} + \dot{x}^T \dot{\lambda}\right) = \left(\frac{\partial H}{\partial u}\right)^T \dot{u}$$

And from (A-7), the time derivative of the Hamiltonian becomes:

$$\frac{dH}{dt} = 0 \quad \text{or} \quad H = \text{constant}$$

From the final condition of the Hamiltonian in (A-12), it can be shown that H = 0.

2. Pontryagin's maximum principle

The principle was developed by the Russian mathematician Pontryagin in the late 1950's.

The principle is wider in scope than the previous section. The principle can be stated as:

$$H\left(x^0, \lambda^0, u^0, t\right) \leq H\left(x^0, \lambda^0, u, t\right) \qquad \text{(A-13)}$$

for all admissible u(t) at all times. This Equation constitutes a necessary condition for the principle. The informal statement may be stated as (Noton, 1972):

In order to minimise the objective functional (A-1), the Hamiltonian must be minimised at all times over all possible u.

3. Necessary and sufficient conditions for the optimal control

As it was mentioned only the necessary conditions, the following additional necessary and sufficient conditions are presented in this section. These conditions are ;

Necessary conditions:

$$H_{uu} \geq 0$$

This condition is so called Legengre-Clebsch Condition. And the strengthened Legengre-Clebsch becomes a sufficient condition.

$$H_{uu} > 0$$

For the Pontryagin's principle, a strong necessary condition is:

$$H\left(x^0, \lambda^0, u, t\right) - H\left(x^0, \lambda^0, u^0, t\right) \geq 0 \; ; \; u \neq u^0$$

This condition is also called the Weierstrass Condition. And the strengthened Weierstrass Condition becomes a sufficient condition.

$$H\left(x^0, \lambda^0, u, t\right) \; - \; H\left(x^0, \lambda^0, u^0, t\right) \; > \; 0 \; ; \; u \; \neq \; u^0$$

Appendix B Model Predictive Control (MPC)

The Model Predictive Control (MPC) can be referred to as the family of controllers in which there is a direct use of an explicit and separately model. The main strategy of model predictive control is to predict the effect of potential control actions on the future values of the process output over a finite interval and find the best control actions which minimise the objective function, which is usually the sum of squared errors between predicted outputs and desired set-points.

The model predictive control has, therefore, two main parameters that need to be chosen by the designer:

1. Prediction horizon: The process models are used to predict the finite future outputs, which are used to compare with the desired set-points.
2. Control horizon: The sequence of finite future control inputs are determined to optimise the objective function based on the finite future outputs and desired set-points.

However only the first control action is implemented and the optimisation procedure is restarted again for the next finite time horizon. This strategy is called moving or receding horizon, which allow the disturbances and plant/model mismatch to be compensated. The receding horizon in the model predictive control is shown in Figure B-1.

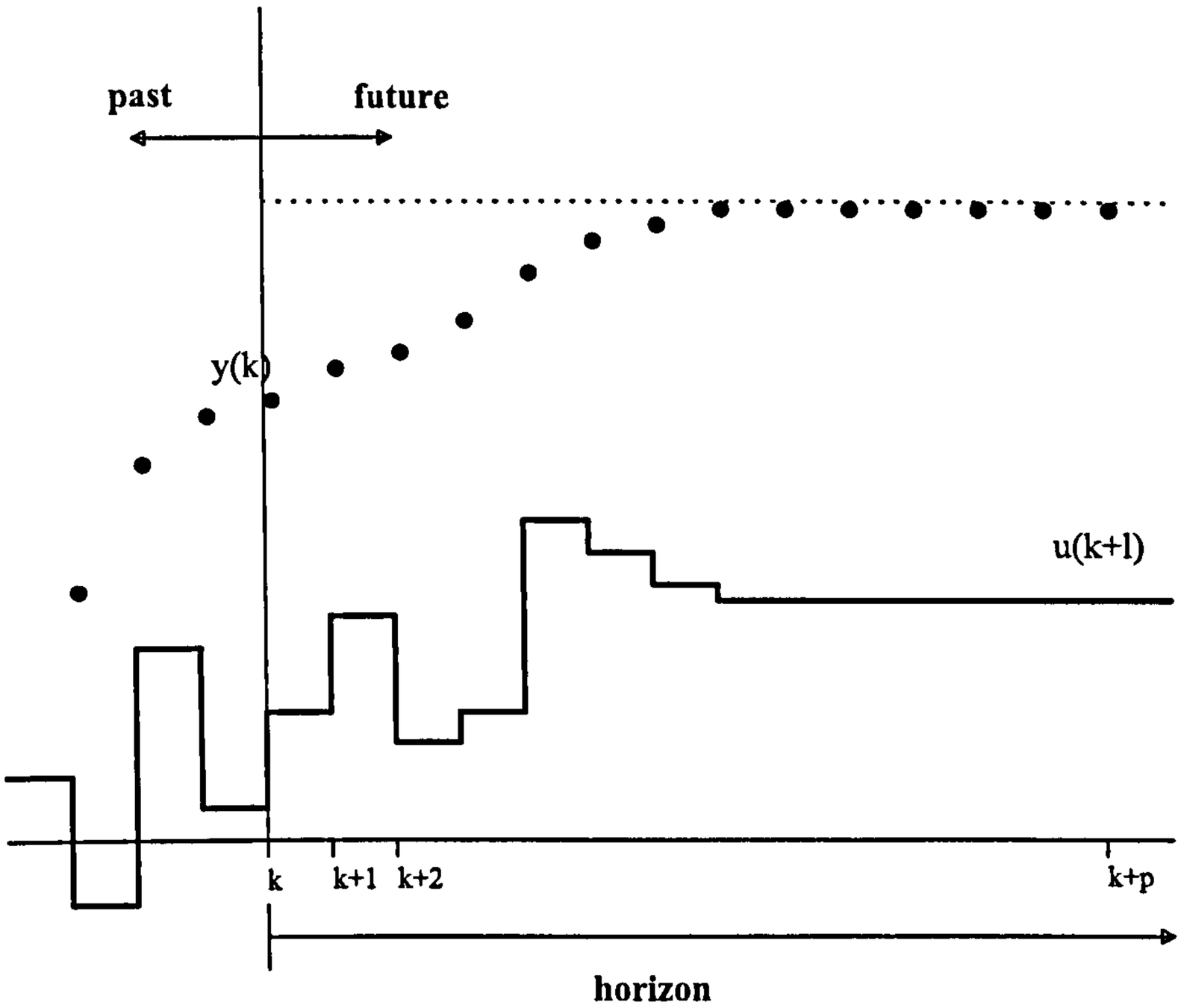

Figure B-1 Receding horizon in model predictive control

The general problem to be solved by the model predictive control can be stated as:

$$\min_{\Delta u(k)\ldots\Delta u(k+m-1)} \quad \sum_{i=1}^{p} \left\| \hat{y}(k+i/k) - r(k+i) \right\|_{\Gamma}^{2} \quad + \quad \left\| \Delta u\big((k+i-1)\big) \right\|_{B}^{2}$$

subject to the system equations, constraints and initial conditions:

$$\frac{dx}{dt} - f(x,u) \;=\; 0$$

$$y - g(x,u) \;=\; 0$$

$$h(x,u) \;=\; 0$$

$$k(x,u) \;\geq\; 0$$

$$x(t_0) \;=\; x_0$$

where

f, g : process model

h : equality constraints

k : inequality constraints

u : input vector

$$\Delta u(k+i) \quad = \quad u(k+i)-u(k+i-1)$$

y : output vector

$\hat{y}(k+i/k)$: model predicted value of y at time (k+i) based on information at time k.

$r(k+i)$: desired set-point at time (k+i).

p : prediction horizon

m : control horizon: $(\Delta u(k+i)=0 \quad \forall i \geq m \quad ; \quad m<p)$

Γ, B : weighting matrices

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EXPERIMENTAL DESIGN FOR PARAMETER ESTIMATION OF BIOCHEMICAL PROCESSES.

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Abstract - Problems of parameter estimation in biochemical processes can be reduced by optimal experimental planning. In this paper, optimal sampling time is used to implement the parameter estimation more accurate with fewer samples. By considering optimal criteria, variance of estimates, as the indication of estimator precision, can be reduced using this strategy.

1. INTRODUCTION

There are several problems in determining the value of parameters in a fermentation process. Problems such as great variability, time variation, nonlinearity and adaptive response of the living microorganism lead to different values of the parameters under different conditions.

Another estimation problem is that some parameters such as maximum specific growth rate and the K constant in the Monod equation have physical meaning and need to belong to prespecified acceptable ranges. These two parameters are usually difficult to identify accurately as shown in Holmberg(1). Therefore specific data is needed for determining the precise value of parameters. Since the parameters are specific random variables, two aspects should be considered. The first is that the expectation of the estimated parameters ($\hat{\theta}$) from infinite time of estimation equals the true parameters (unbiased estimator). The other is that the covariance of the estimated parameters should be very small. This leads to a need for the design of experiments in order to generate data rich in information to help towards the estimation of parameters.

Optimal experimental design, generally, involves in two parts. The first is to determine an objective function on together with its constraints. The other is to optimise the objective function. There are, usually, 4 operation adjustments available for experimental design (4). These are

1. input signal shape
2. sampling rate
3. sampling location
4. filtering of data prior to sampling
5. choice of initial condition
6. choice of variables to perturb
7. choice of variables to sample

2. INFORMATION MEASUREMENT AND SENSITIVITY COEFFICIENT

The measurement of information in data related to the purpose of experiment. For parameter estimation experiment, information data related to parameter accuracy which represented by parameter covariance matrix accomplished the Cramer-Rao lower bound. Hence the

information content can be measured by scalar function of Fisher's information matrix of that system.

Sensitivity coefficient is an important component in Fisher's information matrix. It is formed by taking the first derivative of a dependent variable with respect to a parameter. For optimal input design experiment, this coefficients are maximised via Fisher's information matrix under pre-specified controlled input sequences. Then dependent variables would be as sensitive as possible to the parameters. Inspire of this, covariance of estimator would be minimum and parameter estimation would be more accurate. On sensitivity coefficient itself, it can be used to indicate the information content of data and used for selecting suitable sampling time under the highest period. Sensitivity coefficient is also used to examined whether parameters can be estimated. Parameters can be estimated if the sensitivity coefficients over the range of observation are not linearly dependent.

3. SAMPLING TIME STRATEGIES FOR IMPROVING PARAMETER ESTIMATION.

Consider the kinetic models of batch fermentation process.

$$\frac{dX}{dt} = \mu X \tag{1}$$

$$\frac{dS}{dt} = -\frac{1}{Y}\mu X \tag{2}$$

$$\mu = \frac{\mu_{max} S}{(K+S)} \tag{3}$$

The difficulties of estimating parameters in Monod model usually appear for estimation μ_{max} and K while Y can be estimated not so difficult. Since there is no control input element in batch process, optimal experiment can be performed by choosing suitable sampling time under the certain period. This is very useful when the number of samples are limited and costly.

Optimal Experiment Criteria

To find optimal experiment is to determine the conditions under which each data should be taken in order to optimise some criteria. Variance of an estimator is used generally for measuring its precision. The smaller the variance, the greater the precision. The variance of the estimator is approximate in Beck and Arnold(2) for one parameter as

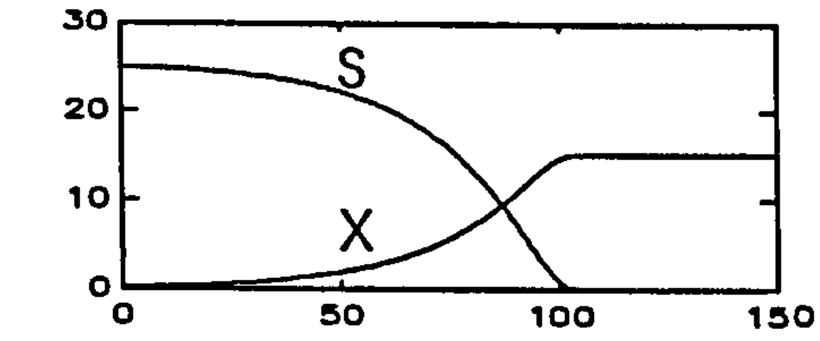
$$V(\theta) = \sigma^2 \left[\sum_{i=1}^{n} X_i^2 \right]^{-1} \tag{4}$$

Where V is variance of estimator, X_i is sensitivity coefficient, and σ^2 is variance of data error. It can be seen that variance of the estimator can be reduced by maximising the value of X_i, or collecting the data at the maximum X_i range. In the same way, covariance is used for multi-parameters and criterion becomes

$$\mathrm{cov}(\theta) = \left(X^T X\right)^{-1} \sigma^2 \tag{5}$$

Which required the data to be taken at maximum $|X^TX|$, while X is sensitivity coefficient matrix in this case.

To show the effect of sampling time to parameter estimation, the simulation data from equation (1), (2) and (3) was drawn in fig.1. including the sensitivity coefficient. After adding white noise, samples were taken for estimating parameters. (Samples would not be taken from the range over 100 since it was in the steady state and no information to be obtained.). The results in table1. showed that the variance of estimates from samples chosen from the high sensitivity coefficient period (range 50-100 and 70-100) have lower value than from sampling taken uniformly from 1-100 hrs (50 and 100 samples) as expected. Comparing 50 and 100 samples in the range 1-100 hrs, 50 samples case was worse than 100 samples case. This was caused by noise effect which made the number of samples not enough for estimation. The noise effect can also be seen from equation (4) and (5) that when σ^2 is bigger, it makes the variance and covariance bigger. However this problem was relieved by choosing the samples at high sensitivity value. (50 samples in the range of 50-100 hrs and 30 samples in the range of 70-100 hrs)



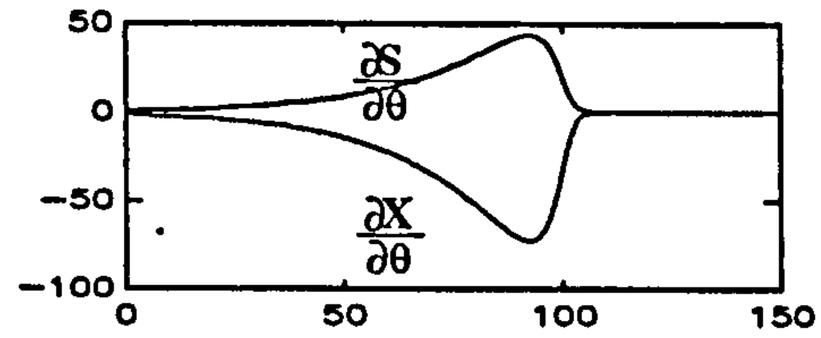


Fig.1 sensitivity of biomass and substrate with respect to parameters

4. CONCLUSION

The optimal sampling time was shown to improve parameter estimation. By this strategy, the number of samples can be reduced which is important in fermentation process while every samples is costly and the number of samples is limited as a constraint in practice. Since priori knowledge is necessary to experimental design. The process knowledge and experience can be useful for designing experiments.

range	1-100		1-100		50-100		70-100	
number of samples	100		50		50		30	
parameter	μ_{max}	K	μ_{max}	K	μ_{max}	K	μ_{max}	K
estimate	0.0497	2.1073	0.1094	3.1002	0.0501	2.0038	0.0514	1.9707
variance	0.00013	5.9885	0.0006	7.3664	7.79E-06	0.1828	5.3E-05	0.0847

The value of $\mu_{max} = 0.05$ and $K = 2$ were used for generating simulation data.

Table1. Estimated value and variance of parameters

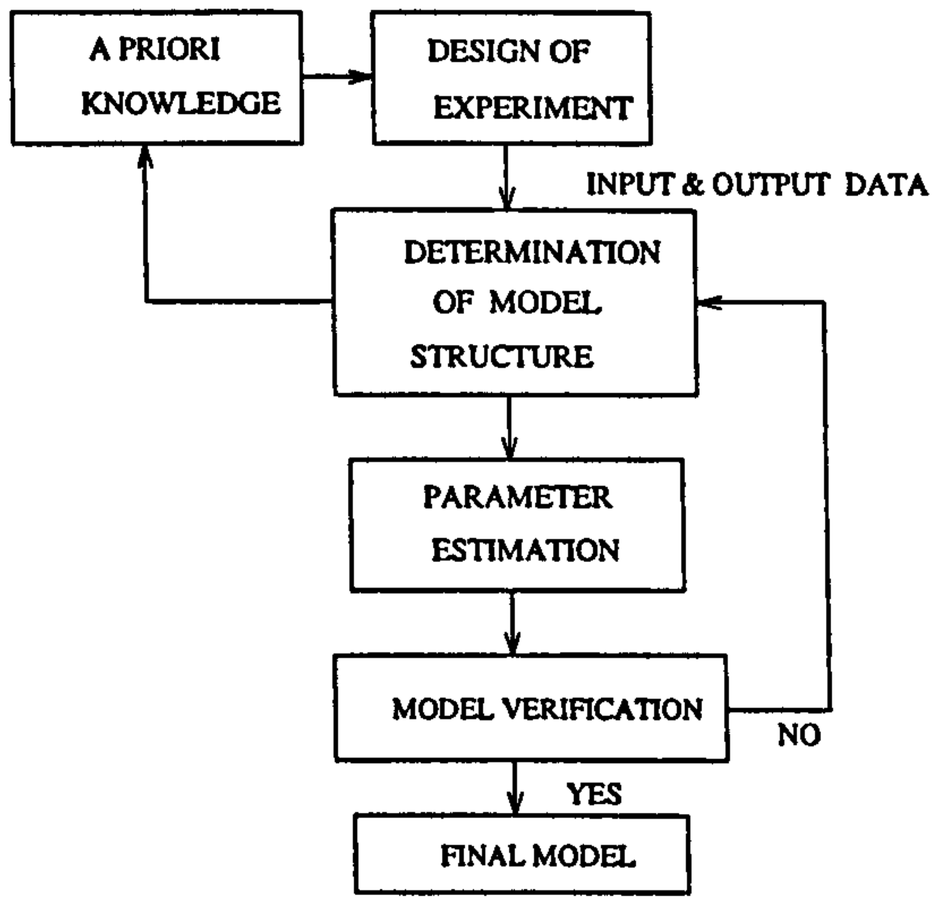


Fig.2 diagram showing experimental design and parameter estimation

5. REFERENCES

1. Holmberg, A., 1982 " On the Practical Identifiability of Microbial Growth Models Incorporating Michaelis-Menten Type Nonlinearities." Math.Biosci. 62, 23-43
2. Beck, J.V. and K.J. Arnold, 1977 Parameter Estimation in Engineering and Science., John Wiley & Sons
3. Bekey, G.A. and S.M. Yamashiro, 1976 " Parameter Estimation in Mathematical Model of Biological Systems. " Adv.Biomed. 6 , 1-43
4. Goodwin, G.C. and R.L. Payne, 1977 Dynamic System Identification : Experiment Design and Data Analysis. Mathematics in Science and Engineering vol. 136 , Academic Press, 1977
5. Johnson, D.B. and P.M. Berthouex, 1975" Efficient Biokinetic Experimental Designs. " Biotech.Bioeng. 17, 557-570
6. Johnson, D.B. and P.M. Berthouex, 1975 " Using Multiresponse Data to Estimate Biokinetic Parameters. " Biotech.Bioeng. 17, 571-583
7. Leigh, J.R., 1983 Modelling and Simulation. Peter Peregrinus Ltd.
8. Mehra, R.K., 1974 " Optimal Input Signals for Parameter Estimation in Dynamic Systems - Survey and New Results." IEEE Tran.Auto.Cont. AC-19(6),753-768
9. Parulekar, S.J. and H.C. Lim, 1985 " Modelling, Optimisation and Control of Semi-Batch Bioreactors. " Adv.Biochem.Eng. 32, 207-258
10. Vialas, C., A. Cheruy and S. Gentil., 1985 " An Experimental Approach to Improve the Monod Model Identification." IFAC Modelling and Control of Biotechnological Process, Noordwijkerhout., 175-179

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MODELLING OF BIOCHEMICAL PROCESSES : A CASE OF COMPLEX MEDIA FERMENTATION

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ABSTRACT

It is well known that conventional biological models can not represent industrial fermentation processes with complex media. In this study, substrate utilisation rate and product formation rate are modelled in the ARMAX structure and in the form of 1 step ahead predictor using available on-line measurements. Model parameters are updated each time when the new data are available. Through recursive approach, for every time interval between samples, new linear time invariant models are obtained which are then updated each time the new data becomes available. By approximation of several linear models during the batch, it is possible to cope with the time varying nature of the process. It is also possible to cope with batch to batch variation resulting from variations in raw materials and operating conditions. The results show small variations in parameters in the models during the batch which may be used to indicate the correct model structure for this process. When the parameters obtained from the recursive approach were used in different batches, without updating parameter values, suitable fitting between predictions and real data was achieved.

INTRODUCTION

Fermentation processes usually suffer from low sampling rate of the desired controlled state variables and time delay due to the laboratory analysis. This results in inefficient control of these processes and needs for models to help prediction of these state variables. Kinetic model for fermentation processes is a mechanistic model based on a priori knowledge of the process which can predict, with high accuracy, the behaviour of the process. However, the incomplete understanding of culture behaviour, the varying growth condition as well as process disturbances affect significantly the accuracy of this model. Hence, there is a need for adaptive schemes for on-line adjustment. For the past several years, a significant amount of research has been devoted to the on-line estimation of the state variables (i.e., biomass, substrate and product concentrations). Assuming known and constant chemical formulae for each species in the reactor, methods based on the elementary balance of C, H, O and N molecules have been established to estimate overall growth. Extended Kalman filter has been adopted to estimate model parameters [1-3] to predict specific growth rate for biomass production. This method is also used in fed-batch penicillin production [4]. However, this is not the case in industrial fermentation processes where complex media with unknown formulae are used and raw materials vary between batch to batch.

This paper investigates an industrial process for microbial secondary metabolite production. The process is operated under fed-batch mode and two main substrates are defined as sub1 and sub2. Sub1 is a complex compound with an unknown formulae. In spite of incomplete knowledge of mechanism of the process, substrates rather than the specific growth rate are used as manipulated variables. In this process, sub1 and sub2 were kept constant at certain limits during the batch. The limits are known from experience as the values for optimal production. Therefore models are needed to estimate these state variables. Due to the characteristics of complex medium in the process, the available limiting substrate concentration can not be measured (although total concentration can be measured) and therefore the Michaelis-Menten equation and its modifications can not be used. Moreover, the elementary balance can not be derived due to unknown substrate formulae. In this paper, the on-line measurements were used to estimate the substrate utilisation-rate and product formation-rate and to have access to unmeasured state variables.

MODELLING

We start with the macroscopic mass balance for this process which can be derived as follow.

$$\frac{dX}{dt} = r_x - D\,X \quad ; \quad r_x = \mu\,X \tag{1}$$

$$\frac{dS}{dt} = r_s + D\,(S_i - S) \quad ; \quad r_s = -\frac{1}{Y_{x/s}}\,\mu\,X \tag{2}$$

$$\frac{dP}{dt} = r_p - D\,P \quad ; \quad r_p = \kappa\,X \tag{3}$$

X = biomass concentration (g/litre)
S = substrate concentration (g/litre) ; refer to as sub1 and sub2

S_i = substrate concentration in feed (g/litre)
P = product concentration (g/litre)
μ = specific growth rate (hr^{-1})
$Y_{X/S}$ = yield substrate to biomass (g biomass / g substrate)
κ = specific product formation rate (hr^{-1})
r_i = reaction rate of species i (g/litre·hr); i = X, S, P

To model X, S, P in these equations, it is possible to model reaction rate (r_X, r_S, r_P). In many cases in industrial processes, biomass concentration can not be measured accurately due to interference by solid substrates. This makes it necessary to bypass the modelling of biomass and specific growth rate and model directly to r_{sub1}, r_{sub2} and r_p. To model r_{sub1}, r_{sub2} and r_p, ARMAX model is adopted in the form of one-step ahead predictor using oxygen uptake rate (OUR), dissolved oxygen (DOT) and respiration quotient (RQ) which can be measured on-line as inputs to the models. Parameters in the models are estimated in recursive manner [5, 6]. The linear model uses available information up to time t to predict reaction rate (r_i) at time t + 1 and obtains an upgraded model during this period. When the new data are available, the model is updated and the prediction is repeated in the same manner. By this method, time varying aspect of the process can be represented by several linear models along the batch.

RESULT AND DISCUSSION

The results of model prediction compared with real data of r_{sub1}, r_{sub2} and r_p are shown in fig. 1, 3 and 5 with the model parameters in fig. 2, 4 and 6. Since the batches are operated at the same operating condition, the suitable initial parameters of the models can be obtained after testing on several batches. The results show a small variation in the parameters which indicates the suitable model structure in this case. These parameters, then, can be used without a need for recursive updating as shown in fig. 7 and 8 for prediction of r_{sub1} and r_{sub2} in another batch. The results also show a good fit for r_p (not shown here). However, the intention of using recursive method is to cope with the time varying aspect of the fermentation process. This means that even when the operating conditions or raw materials change, this method can still be used. However, there are limitations. First, this method needs off-line measurements to update the models. During that period, the models obtained are linear time-invariant. This is reasonable in fermentation processes which have slow dynamic. Second, the sampling period limits the model accuracy. The shorter the sampling period, the better the model prediction. The dynamics of the process as well as cost of sampling have to be considered when choosing a suitable sampling time. When substrate utilisation in the fermentation process is known, substrate concentration can be calculated and optimal feed profile can be obtained to control the substrate concentration at the desired level.

REFERENCES

1 Stephanopoulos, G., & San, K.-Y., 1984 Biotech.Bioeng. 26, 1176-1188.
2 Stephanopoulos, G., & San, K.-Y., 1982 On-Line Estimation of Time-Varying Parameters. Application to Biochemical Reactors. In IFAC Modelling and Control of Biotechnical Processes, 195-199. Helsinki, Finland:
3 San, K.-Y., & Stephanopoulos, G., 1984 Biotech.Bioeng 26, 1209-1218.
4 Mou, D.-G., & Cooney, C. L., 1983 Biotech.Bioeng 25, 225-255.
5 Ljung, L., & Soderstrom, T., 1985. Theory and Practice of Recursive Identification. The MIT Press.
6 Ljung, L., & Gunnarsson, S., 1990 Automatica 26(1), 7-21.

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Fig. 1 Sub1 utilization rate

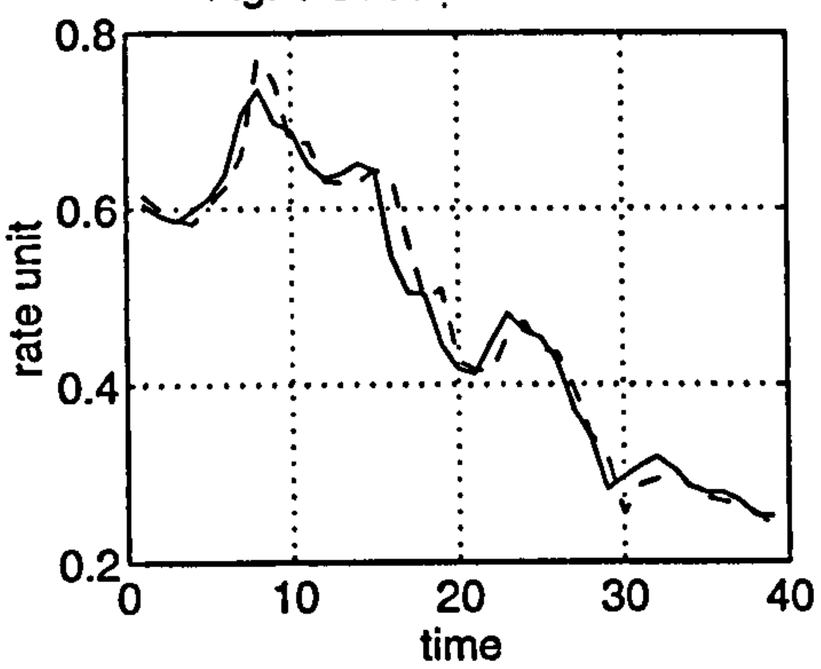


Fig. 2 Parameters in sub1 utilization model

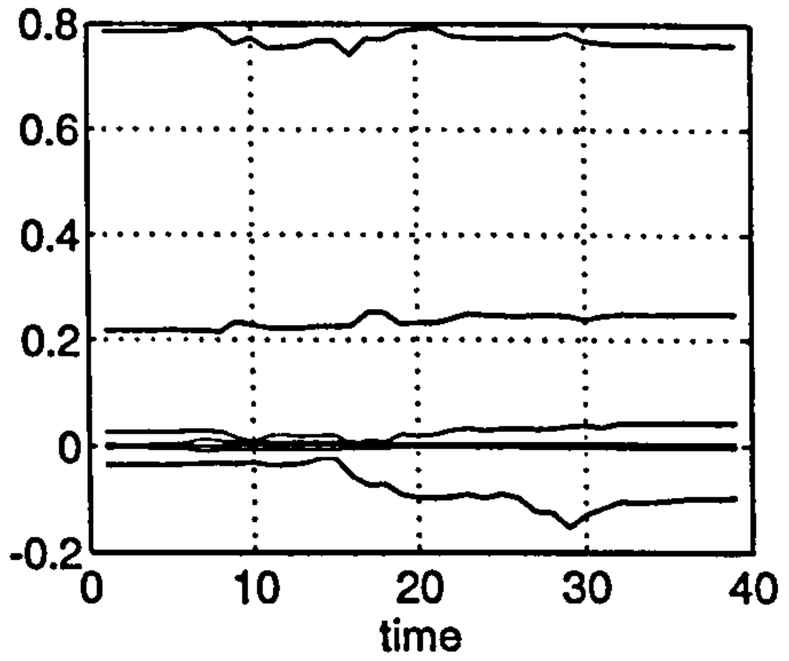


Fig. 3 Sub2 utilization rate

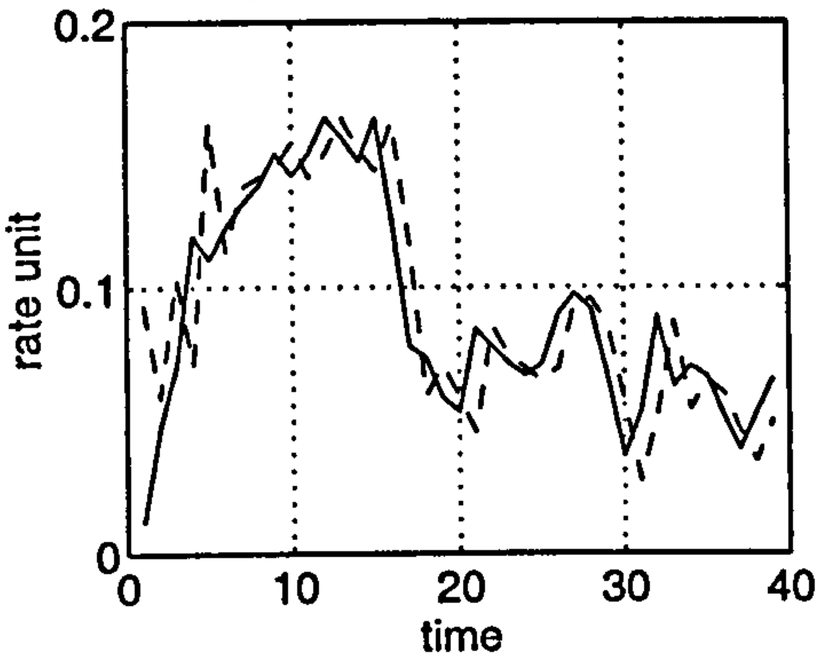


Fig. 4 Parameters in sub2 utilization model

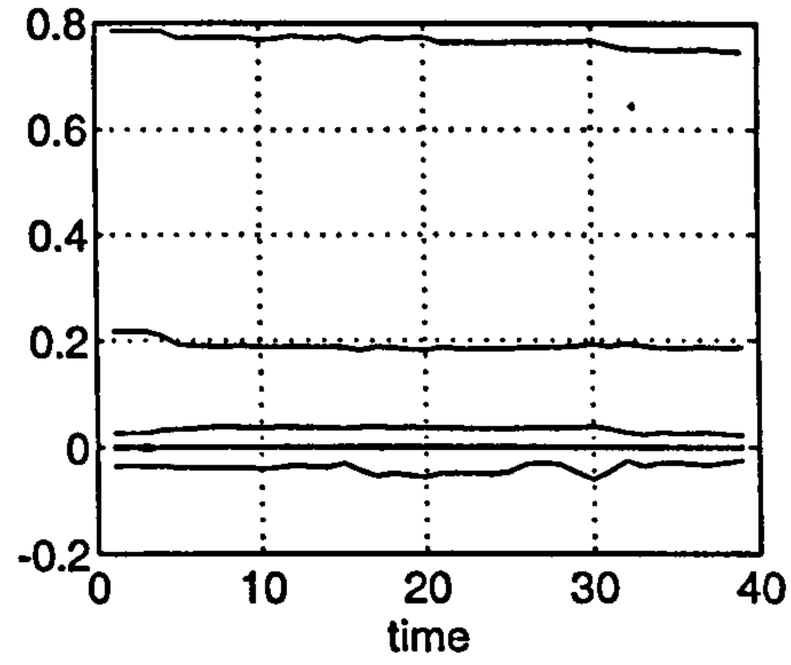


Fig. 5 Product formation rate

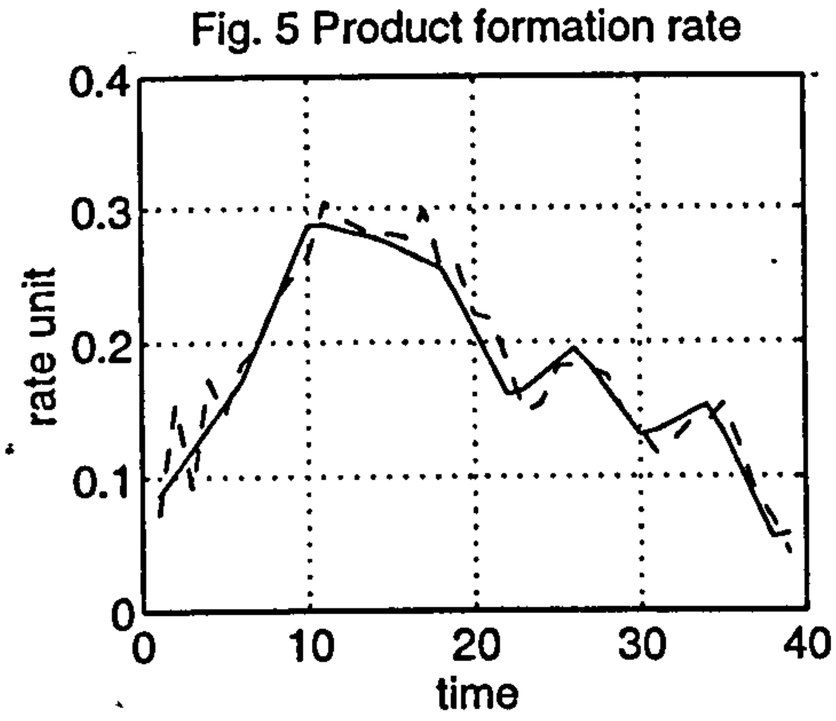


Fig. 6 Parameters in product formation model

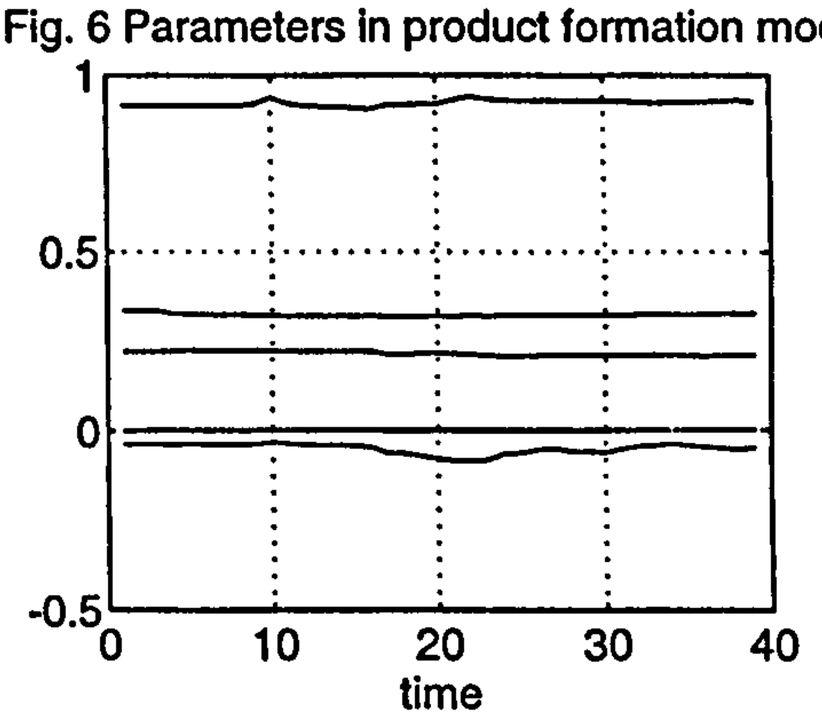


Fig. 7 Sub1 model without updating parameters

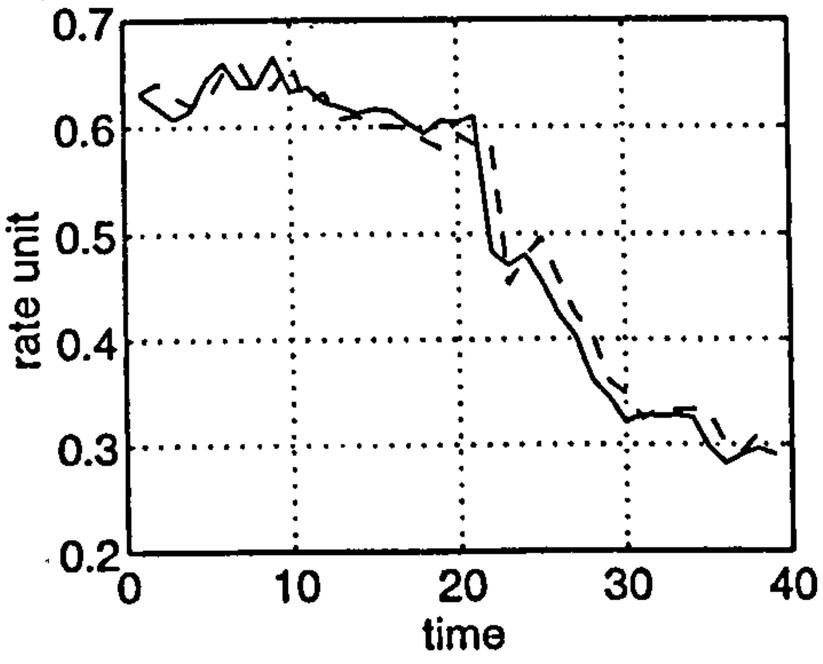
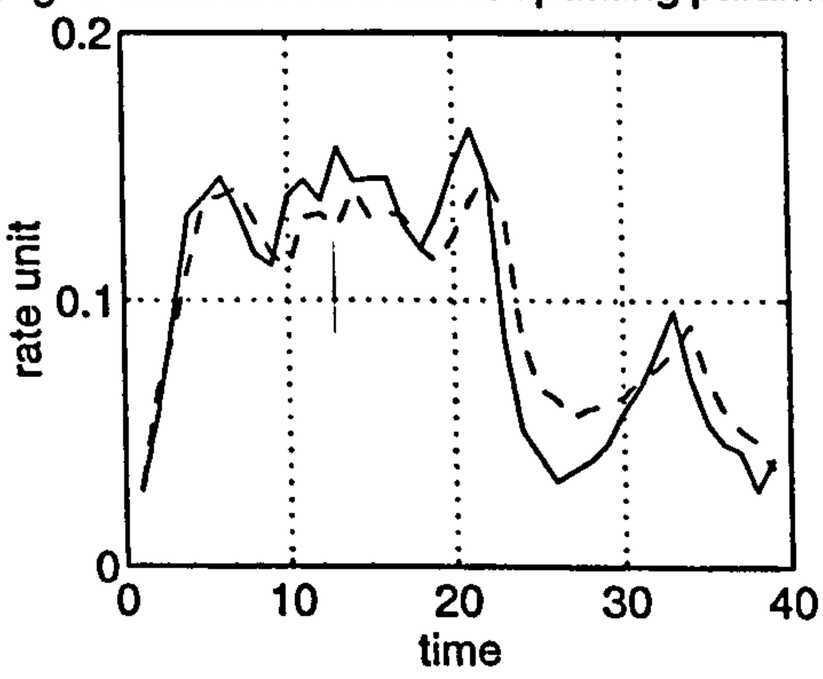


Fig. 8 Sub2 model without updating parameters



' ——— ' solid lines represent data

' -------- ' dashed lines represent prediction

Comparison of Open Loop Optimal Control and Closed Loop Optimal Control of a Fermentation Process.

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Abstract

Optimisation of a fed-batch fermentation process is usually done by using the calculus of variations to determine an optimal feed rate profile. This often results in a singular control problem and an open loop control structure. To overcome these problems, the closed loop optimal control is developed by dividing the optimisation problem into two parts. First, an optimal substrate concentration profile which has direct effect to the biochemical reactions in the fermentation process is derived. Then a controller is designed to track the obtained optimal profile. A biomass production process is used to compare the performance of the closed loop optimal control method and the open loop optimal feed rate control method. The results show a better performance of the closed loop optimal control than the open loop optimal feed rate profile. This is due to the feedback property of the closed loop optimal control method.

Keyword : process control, optimisation, fermentation process

1. Introduction

Fermentation processes are used for producing many fine chemical substances such as amino acids, antibiotics, biomass, enzymes, etc. From modes of operation, (batch, fed-batch and continuous), fed-batch operation is often used in industry due to its ability to overcome the catabolite repression or glucose effect, which usually occur during production of these fine chemicals [1, 2]. Moreover, it also gives the operator the freedom of manipulating the process via substrate feed rate. This gives the challenge to the control and optimisation of the fed-batch fermentation processes.

Optimisation of fed-batch fermentation processes have been a topic of research for many years. To determine an optimal feed rate profile in the fed-batch fermentation, the other environment variables such as temperature and pH, which also affect bioreaction rates in the processes are assumed constant at some levels. The approaches used by many research groups to determine the substrate feed rate profile that optimises a desired objective function are usually based on the calculus of variations [3-8] or Green function [9]. And since there are physical constraints in the minimum and maximum feed rates, the Pontryagin's Maximum principle is applied. However, there are two problems arising in applying the variational method to the fermentation process. The first is that a singular control situation may occur during operation since the control input or the substrate feed rate appears linearly in the Hamiltonian. The other problem is that the obtained optimal feed rate profile from the variational method is in the open loop manner and can suffer quite severely when the model parameters are not exact when used in the real application. It is not until recently that a reliable neural network-based estimation of the substrate concentration has been developed and successfully implemented in industry [24] that we are ready to proposed a method that can avoid these problems [10]. The method separates the optimisation problem of the fermentation process into two parts. Firstly, the optimal substrate concentration profile which has direct effect to the biochemical reactions in the fermentation process is derived. Then a controller is designed to track the profile of the obtained optimal substrate concentration. With this approach, the singular problem is overcome, as the substrate concentration usually appears as a nonlinear function in the Hamiltonian. The open loop control is also converted into a close loop servo or regulator control. This method is then called here the "closed loop optimal control". The objective of this paper is to demonstrate the advantages of the closed loop optimal control method

comparing to the open loop optimal feed rate control method.

The paper is structured as follows: Section 2 will describe the mathematical representation of a fed-batch fermentation process and formulate the closed loop as well as the open loop optimal control. The comparison between both methods is then shown in Section 3. The comparison is concluded in Section 4.

2. Closed Loop Optimal Control

The fed-batch fermentation can be represented by the following dynamic mass balance equations.

$$\frac{dX}{dt} = \mu X - D X \quad (1)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{xs}} \mu X + D\left(S_f - S\right) \quad (2)$$

$$\frac{dP}{dt} = \pi X - D P \quad (3)$$

$$\frac{dV}{dt} = F \quad (4)$$

$$D = F/V \quad (5)$$

where X, S, P are biomass, substrate and product concentration (g/l) in the reactor respectively; F is the substrate feed rate (l/hr.); S_f is the concentration of substrate in the feed stream (g/l); D is dilution rate (1/hr.); μ and π are specific cell growth rate and specific product formation rate respectively (1/hr.); Y_{xs} is the yield of cell mass from substrate (g cell/g substrate) and V is fermenter volume (l). The specific rates μ and π are functions of substrate concentration. Further details and analysis on the fed-batch operation can be found in [2, 11, 12].

The fed-batch fermentation is constrained by conditions on final volume and minimum and maximum of substrate feed rates:

$$0 \leq F \leq F_{max} \quad (6)$$

$$V\left(t_f\right) = V_f \quad (7)$$

The objective of the fermentation process is to produce as much product as possibly under production-time constraint. This objective is transformed into an objective function shown in Equation (8) and can be solved using the calculus of variations [13-15].

$$J(F) = f\left(X\left(t_f\right), P\left(t_f\right)\right) \quad (8)$$

The obtained open loop optimal feed rate profile consists of a sequence of maximum, minimum and singular feed rates depending on the following condition:

$$\frac{\partial H}{\partial F} = -\frac{\lambda_X X}{V} + \lambda_V + \frac{\lambda_S\left(S_f - S\right)}{V} - \frac{\lambda_P P}{V} = \Psi$$

From the Maximum principle, the optimal feed rate is determined by Ψ as follow:

if $\Psi < 0$ then $F = 0$

if $\Psi > 0$ then $F = F_{max}$

if $\Psi = 0$ then $F = F_{sing}$

The singular feed rate can be determined by repeatedly differentiating Ψ until feed rate (F) appears in the time derivative equation of Ψ.

$$\frac{d^k}{dt^k}\Psi = 0 \quad ; \quad k = 1, 2, 3, \ldots$$

The closed loop optimal control method separates the optimal control into two parts. The first part is to determine the optimal substrate concentration using the calculus of variations method but change the control variable from the substrate feed rate to substrate concentration in the fermenter. This eliminates the singular problem occurred in the open loop optimal feed rate profile method.

A controller can then be designed to track the obtained optimal substrate concentration profile and the control problem becomes a closed loop control problem. This also offers the flexibility that many types of controllers can be designed and used particularly those robust to model/process mismatch errors [16].

To determine the optimal substrate profile, the substrate feed rate and volume are omitted and the system equations become:

$$\frac{dX}{dt} = \mu X \quad (9)$$

$$\frac{dP}{dt} = \pi X \quad (10)$$

The objective function is then changed to:

$$J(S) = f\left(X\left(t_f\right), P\left(t_f\right)\right) \quad (11)$$

The optimal control can then be written down as:

$$\frac{\partial H}{\partial S} = \lambda_X X \frac{\partial \mu}{\partial S} + \lambda_P X \frac{\partial \pi}{\partial S} = 0 \quad (12)$$

And the optimal substrate concentration profile can be obtained by solving Equation (12) for the substrate concentration (S).

We use a nonlinear model predictive control scheme for the tracking control since the process model is available and nonlinear. More details on nonlinear model predictive control are in [17-23].

3.Comparison of open loop optimal feed rate profile (OLOFP) and closed loop optimal control (CLOC)

A biomass production process is used for demonstration and comparison between both methods. The objective of the process is to maximise the biomass production as shown in (13). The specific growth rate is assumed to be the substrate inhibition kinetic as shown in (14). The substrate inhibition kinetic is used in this study not only because it is simple to understand but also it can provide an analytical solution for comparison. Moreover, this type of kinetic can represent the catabolite repression and therefore the need for operating the fermentation in the fed-batch mode. Note that the model for this process consist of Equation (1), (2), (4) and (5).

$$J(F) = \max X(t_f) \qquad (13)$$

$$\mu = \frac{\mu_{max} S}{\left(K_S + S + S^2/K_i\right)} \qquad (14)$$

It was shown in [10] that the optimal substrate concentration under the substrate inhibition kinetic can be calculated from the following condition, which is also the condition that the singular period occurs in the open loop optimal feed rate control:

$$\mu' = \frac{d\mu}{dS} = 0 \qquad (15)$$

or

$$S_{opt} = \sqrt{K_S \cdot K_i}$$

For the open loop optimal feed rate profile method, the following feed rate can be obtained during the singular period:

$$F_{sing} = \frac{\mu X V}{Y_{xs}\left(S_f - S_{opt}\right)} \qquad (16)$$

We consider here only the period in which the singular period occurs and therefore the substrate concentration is kept at the optimal from the beginning of the batch.

The following parameter values are used in the simulation:

$\mu_{max} = 0.10$, $K_s = 3$, $K_i = 8.34$ and $Y_{xs} = 0.164$
initial condition : $X(0) = 1$ g/l, $S(0) = 5$ g/l, and $V(0) = 20$ l
Final condition : $V(t_f) = 50$ l
substrate concentration in the feed stream:
$S_f = 100$ g/l
optimal substrate concentration:
$S_{opt} = \sqrt{K_S \cdot K_i} = 5$ g/l

In the closed loop optimal control, nonlinear model predictive control is used with the following parameters:

sampling time - 1 hr.
prediction horizon - 5 hr.
control horizon - 3 hr.

Since the CLOC method use the same process model as the OLOFP method to determine the optimal substrate concentration profile and feed rate, for an exact process model case, both methods give the similar results [10]. The comparison simulation here will therefore be performed under the condition of process-model mismatch where a small error (10%) is introduced to the parameter Y_{xs} to demonstrate this situation.

The simulation results for the OLOFP and CLOC methods are shown in Figure 1. It can be seen that in the modelling error case, the OLOFP method gives a wrong determination of the optimal feed rate. This results in the accumulation of substrate concentration in the fermenter which in turn reduces the growth rate and prolongs the operating time up to 85 hr. In the CLOC case, the substrate concentration is maintained at the optimal at 5 g/l for the whole batch until the reactor is full. The operating time for the OLOC method is only 72 hr.

The better performance of the CLOC than the OLOFP can be explained from the fact that the optimal singular feed rate needs accurate values of Y_{xs} and S_f to calculate the optimal feed rate as shown in (16), while the error in these two values can be compensated by feedback information in the CLOC method. Improvement of the CLOC method over the OLOFP method at different levels of error in the parameter Y_{xs} is shown in Figure 2.

Note that in both methods, the optimal and singular substrate concentration is determined by parameter K_s and K_i as shown in (15). Therefore, if these two parameters are inaccurate, both methods would result in the incorrect optimal substrate concentration profile and incorrect optimal feed rate profile.

However, the performance of the CLOC method is still better than the OLOFP method in these cases as shown from the shorter operating time in Table 1 where an error in parameter K_s is used for illustration. In this example, it is assumed that parameter K_s in the model equals 3, while this real parameter in the process equals 2 and 4. The incorrect optimal substrate concentration that is calculated from the model (K_s = 3) is 5 g/l while the true optimal levels are 4.083 g/l (K_s = 2) and 5.774 (K_s = 4).

It is shown in the table that for the process with K_s = 2, the operating time if the parameter in the model is correct is 68 hours. The operating time for the CLOC method is 68.5 hours, which is a little longer than the true one but shorter than that obtained from the OLOFP method, which is at 74 hours. For the process with K_s = 4, the operating time if the parameter in the model is correct is 81 hours. The operating time for the CLOC method is 82 hours and that obtained from the OLOFP method is 97 hours. The better performance of the CLOC method can be explained by the fact that keeping the substrate concentration at the incorrect optimal level (5 g/l) decreases the specific growth rate slightly from the optimal growth rate. While in the OLOFP method, the error in parameter K_s results in deviation of the optimal feed rate and substrate concentration, which in turn have highly effect on the biomass growth rate and prolong the process operating time.

This illustration shows the advantage of the CLOC method over the OLOFP method even in the case of incorrect parameter that is used in the optimal substrate concentration determination.

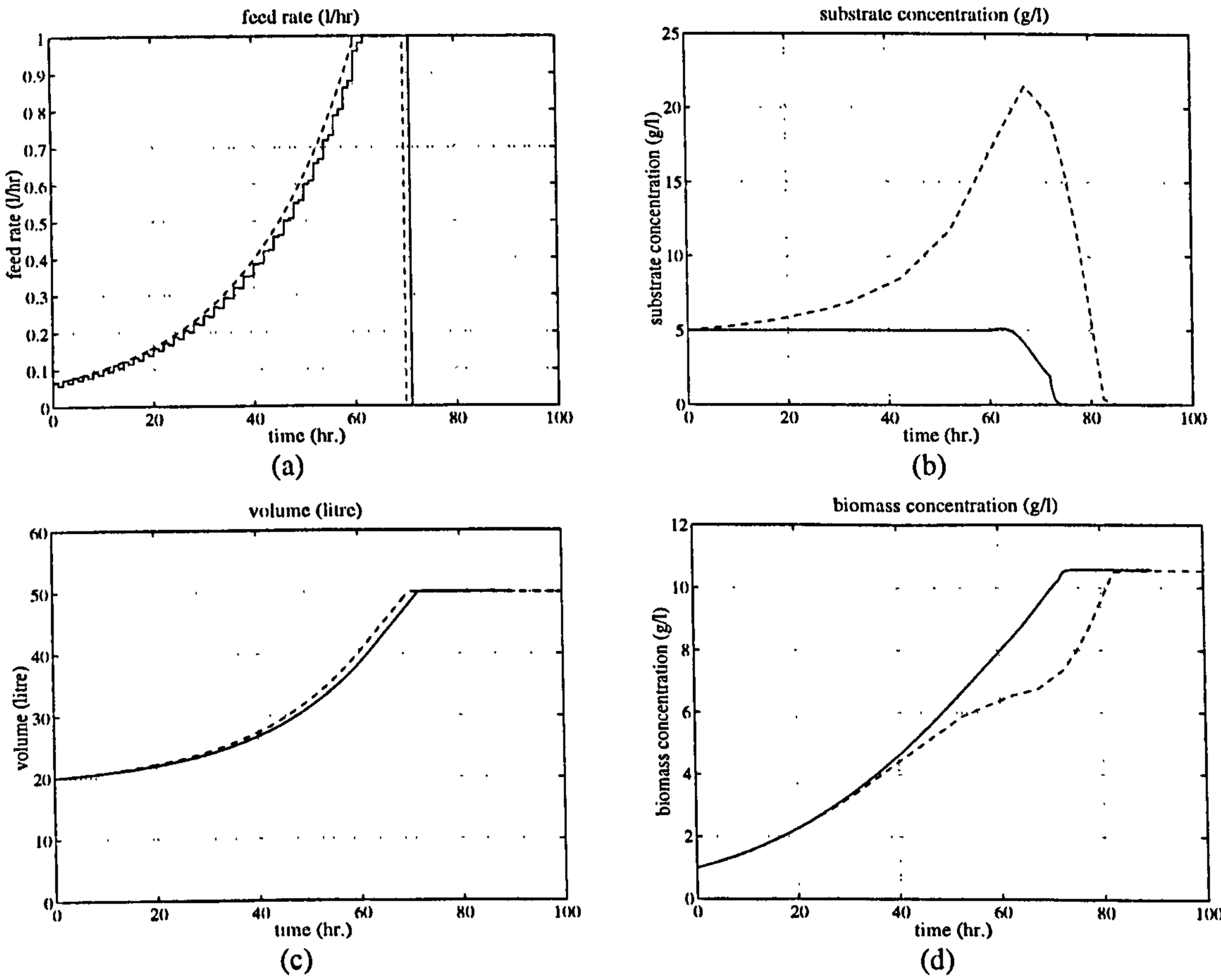

Figure 1 Comparison for both methods for plant/model mismatch in a biomass production process.

Solid line: closed loop optimal control method

Dashed line: open loop optimal feed rate profile method

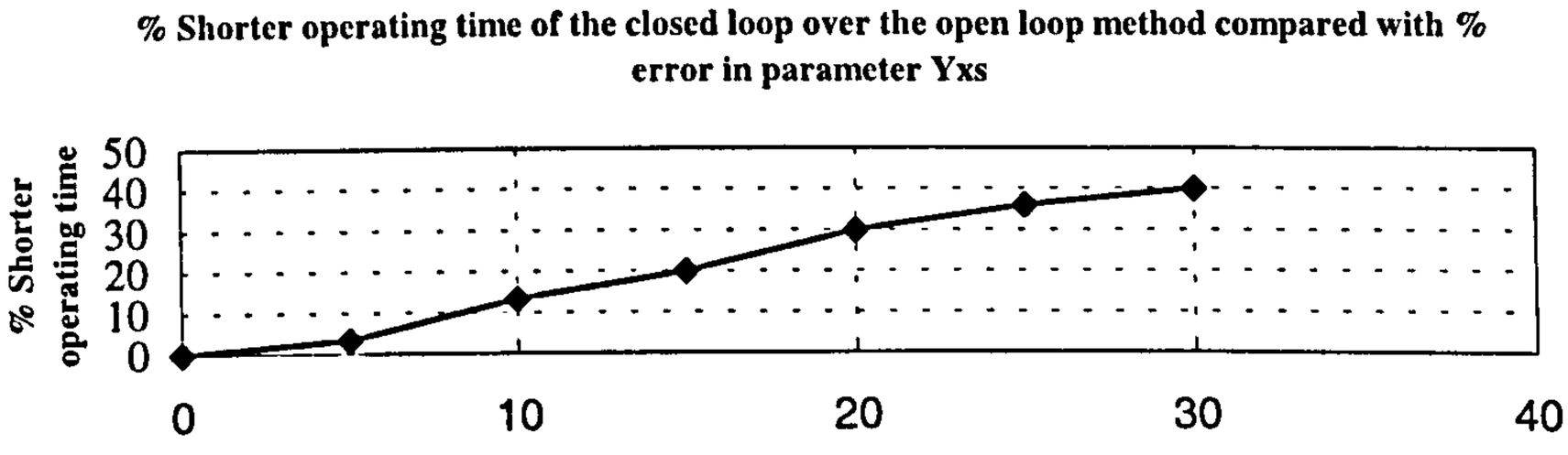

Figure 2 The closed loop method results in shorter operating time than the open loop method in the plant-model mismatch case.

Table 1 Effect of incorrect parameter K_s to the process operating time

Parameter K_s	Operating time (hr.)		
	Correct parameter for both methods	Incorrect parameter (K_s = 3)	
		CLOC	OLOFP
2 (-50%)	68	68.5	74
4 (25%)	81	82	97

4. Conclusion

The closed loop optimal control method divides an optimisation problem in a fed-batch fermentation into two parts.

1. determination of an optimal substrate concentration profile.
2. design a controller to follow the obtained optimal substrate concentration profile.

With this two steps procedure, the singular problem is overcome. And by converting the open loop into closed loop, it was shown by an example on a biomass production process that the closed loop optimal control provides a better results than the open loop optimal feed rate control method. This is due to the feedback property that provide the feedback information to compensate for the modelling error.

5. Reference

1. Parulekar, S.J. and H.C. Lim, "Modeling, optimization and control of semi-batch bioreactors." *Adv.Biochem.Eng.*, 1985. **32**: p. 207-258.

2. Yamane, T. and S. Shimizu, "Fed-Batch Techniques in Microbial Processes." *Adv.Biochem.Eng.*, 1984. **30**: p. 148-192.

3. Weigand, W.A., H.C. Lim, C.C. Creagan, and R.D. Mohler, "Optimization of a Repeated Fed-Batch Reactor for Maximum Cell Productivity." *Biotech.Bioeng.Symp.*, 1979. **9**: p. 335-348.

4. Cazzador, L., "On The Optimal Control of Fed-Batch Reactors with Substrate-Inhibited Kinetics." *Biotech.Bioeng.*, 1988. **31**: p. 670-674.

5. Modak, J.M., H.C. Lim, and Y.J. Tayeb, "General Characteristics of Optimal Feed Rate Profiles for various Fed-Batch Fermentation Processes." *Biotech.Bioeng.*, 1986. **28**: p. 1396-1407.

6. Lim, H.C., Y.J. Tayeb, J.M. Modak, and P. Bonte, "Computational Algorithms for Optimal Feed Rates for a class of Fed-Batch Fermentation : Numerical Results for Penicillin

and cell Mass Production." *Biotech.Bioeng.*, 1986. **28**: p. 1408-1420.

7. Takamatsu, T., S. Shioya, and Y. Okada, "Profile Control Scheme in a Bakers' Yeast Fed-Batch Culture." *Biotech.Bioeng.*, 1985. **27**: p. 1675-1686.

8. Shimizu, H., K. Araki, S. Shioya, and K.-I. Suga, "Optimal Production of Glutathione by Controlling the Specific Growth Rate of Yeast in Fed-Batch Culture." *Biotech.Bioeng*, 1991. **38**: p. 196-205.

9. Ohno, H., E. Nakanishi, and T. Takamatsu, "Optimal control of a semibatch fermentation." *Biotech.Bioeng.*, 1976. **18**: p. 847-864.

10. Vanichsriratana, W., D.C. McFarlane, T. Keshavarz, and J.R. Leigh, "Optimal Control of Fed-Batch Fermentation Processes." 1996. To be submitted.

11. Dunn, I.J. and J.-R. Mor, "Variable-Volume Continuous Cultivation." *Biotech.Bioeng.*, 1975. **17**: p. 1805-1822.

12. Lim, H.C., B.J. Chen, and C.C. Creagan, "An Analysis of Extended and Exponentially-Fed-Batch Cultures." *Biotech.Bioeng.*, 1977. **19**: p. 425-433.

13. Noton, M., *Modern Control Engineering*. 1972, Pergamon Press.

14. Bryson, A.E. and Y.C. Ho, *Applied Optimal Control*. 1975, Hemisphere.

15. Ramirez, W.F., *Process Control and Identification*. 1994, Academic Press.

16. Green, M. and D. Limebeer, *Robust Control Systems*. 1994, Prentice Hall

17. Sistu, P.B. and B.W. Bequette, "Nonlinear Predictive Control of Uncertain Processes: Application to a CSTR." *AIChE.J.*, 1991. **37**(11): p. 1711-1723.

18. Biegler, L.T. "Optimization Approaches to Nonlinear Model Predictive Control." in *Chemical Process Control CPCIV. Proceedings of the Fourth International Conference on Chemical Process Control*. 1991. Padre Island, Texas: CACHE, AIChE.

19. Ali, E. and E. Zafiriou, "Optimization-based Tuning of Nonlinear Model Predictive Control with State Estimation." *J.Proc.Cont.*, 1993. **3**(2): p. 97-107.

20. Cuthrell, J.E. and L.T. Biegler, "On the Optimization of Differential-Algebraic Process Systems." *AIChE J.*, 1987. **33**(8): p. 1257-1270.

21. Renfro, J.G., A.M. Morshedi, and O.A. Asbjornsen, "Simultaneous Optimization and Solution of Systems Described by Differential/Algebraic Equations." *Comp.Chem.Eng.*, 1987. **11**(5): p. 503-517.

22. Eaton, J.W. and J.B. Rawlings, "Feedback Control of Chemical Processes Using On-Line Optimization Techniques." *Comp.Chem.Eng.*, 1990. **14**(4/5): p. 469-479.

23. Eaton, J.W. and J.B. Rawlings, "Model-Predictive Control of Chemical Processes." *Chem.Eng.Sci.*, 1992. **47**(4): p. 705-720.

24. Zhang, B.S., R. Tang, J.R. Leigh, K.D. Dixon, and R.D. Hinge,. "Improved Quality and Productivity in Secondary Metabolite Fermentation through Estimation Control and Scheduling", in *Proc. 13th IFAC World Congress*. 1996. San Francisco