Bioprocess Engineering 21 (1999) 77–88 © Springer-Verlag 1999

# Control of fermenters – a review

**K. Yamuna Rani, V.S. Ramachandra Rao**



**Abstract** Fermenter control has been an active area of research and has attracted more attention in recent years. This is due to the new developments in other related areas which can be exploited to overcome the inherent difficulties in fermenter control. Beginning with conventional regulatory control of operating variables such as temperature, pH and dissolved oxygen concentration, research in fermenter control has undergone significant changes including the recent neural network based approaches. The objective of the paper is to focus the attention of the researchers to the developments in the control of batch, fed-batch and continuous fermenters over the past few years.

## 1 Introduction

The biotechnology industry is evolving rapidly. Many biotechnology-based products such as pharmaceutical and health-care products, agricultural products, and chemicals have already been commercialised. The ability to control a fermentation process at its optimal states precisely and automatically is now of considerable interest to many fermentation industries. Proper control enables them to reduce their production costs and increase the yield while at the same time maintaining the quality of the desired product. During the past few decades, control has been used in the fermentation industry to maintain the operating temperature, pH and dissolved oxygen (DO) concentration at the desired level. However, the level of sophistication in the definition of control objectives as well as the design of control schemes has not yet reached that found in the chemical industry.

Biochemical processes are difficult to control. This is due to the need for precise control resulting from the sensitivity of the micro-organisms and the inability to fully influence the cells' internal environment by manipulating the external environment in which they live. The main factors contributing to the difficulty in control of fermenters are: (1) Their dynamic behaviour is inherently nonlinear, (2) Accurate process models are rarely available due to the complexity of the underlying biochemical processes, (3) Model parameters vary in an unpredictable manner and (4) Reliable biosensors to measure intercellular activities are rarely available, making the process states very difficult to characterise. Bogle et al. [1] discussed the techniques of metabolic pathway engineering which help to identify how to modify the micro-organisms for improved process performance through better design leading to less sensitivity to load changes.

Three modes of operation are very common in the operation of fermenters-batch, continuous and fed-batch. During batch operation of a process, no substrate is added to the initial charge nor is the product removed until the end of the process. Some pharmaceutical preparations are made in this way, but generally batch operation is not commercially attractive. More economic is the continuous operation where substrate is continually added and product continually removed. Examples are the continuous fermentation of milk in the production of margarine and the biological purification of waste water. In fed-batch operation, the feed rate may be changed during the process but no product is removed until the end. Baker's yeast and antibiotics, such as penicillin are made in fed-batches commercially, and there is an enormous economic incentive to optimize such processes [2].

Modak and Lim [3] investigated several modes of operation of bioreactors for fermentation processes like continuous, single-cycle batch, repeated batch, single-cycle fed-batch and repeated fed-batch. They stated that for maximizing bioreactor productivity, continuous operation is a better choice over other options. They classified fermentation processes into four types A, B, C and D depending on whether the instantaneous metabolite yield (defined as the ratio of specific metabolite production rate to specific consumption rate) increases, decreases, goes through maxima, or remains constant, respectively, with increase in substrate concentration. The optimal mode of operation of each of these types is reported as single-cycle batch for type A, continuous for type B, single-cycle fed-

*Received: 17 August 1998*

K. Yamuna Rani
Process Dynamics and Control Group,
Chemical Engineering Division,
Indian Institute of Chemical Technology,
Hyderabad 500 007, India
e-mail: kyrani@iict.ap.nic.in

V.S. Ramachandra Rao
Department of Chemical Engineering,
Indian Institute of Technology,
Madras 600 036, India
e-mail: vsrr@acer.iitm.ernet.in

*Correspondence to*: K. Yamuna Rani

Advice and suggestions from Dr. A.A. Khan are gratefully acknowledged.

IICT Communication Number 4121

batch for type C, and any repeated batch operation for type D processes. They further stated that the optimal results may merely represent theoretical limits in cases where they are not physically realisable.

The control objective in a fermentation process is to maximize the production of a desired product. For batch and fed-batch fermenters, this amounts to maximizing the quantity of the desired product at the end of the batch. This requirement leads to a dynamic optimization problem that is often difficult to solve. For continuous fermenters, the amount of desired product produced per unit time, namely the productivity, can be maximized by optimizing the steady-state operating conditions of the fermenter, which requires solving a steady-state optimization problem.



## 2
## Continuous fermenters

In most of the continuous fermentation processes, one of the output variables is chosen as the controlled variable and its estimated optimal open-loop profile or a constant setpoint is tracked. Menawat and Balachander [4] proposed alternate control structures for maintaining a constant biomass concentration ($X$) in a chemostat, and claimed that the control scheme which employs feed substrate concentration ($S_f$) is more effective than the one which employs dilution rate ($D$) as the manipulated variable. Zhao and Skogestad [5] compared various control configurations for continuous bioreactors. Five control configurations have been investigated, namely conventional turbidostat ($D - X$), conventional nutristat ($S_f - X$), concentration turbidostat ($D$ − substrate concentration $S$) concentration nutristat ($S_f - S$), and modified turbidostat (dilution rate of sterile water stream, $D_w - X$) by using simple proportional-integral (PI) controllers on the basis of partial disturbance gain used for evaluating their controllability with respect to disturbance rejection. At substrate limited growth conditions, the concentration turbidostat is reported to be the best control configuration, whereas conventional turbidostat should be avoided. When the cell growth is not substrate limited, all the control configurations are effective except concentration nutristat which is unacceptable at all operating points because $S_f$ has no steady-state effect on $S$.

### 2.1
### Adaptive control approaches

Adaptive control techniques were one of the earliest advancements implemented in continuous fermenter control. Dochain and Bastin [6] proposed simple self-tuning type nonlinear adaptive controllers for bacterial growth systems in the form of two strategies, namely substrate concentration control and production rate control. The special feature of their application is that the identified parameters of growth rate and yield coefficient have a clear physical meaning and can give in real-time, useful information on the state of biomass. Renard et al. [7] successfully implemented a simple adaptive nonlinear model based control to a biomethanation pilot reactor. Effluent substrate concentration in the anaerobic digester is controlled with the help of dilution rate without using any mathematical description of the specific growth rate. Suarez-Cortez et al. [8] designed a sliding controller for regulation of substrate concentration in a continuous culture fermentation process. Recently, Dochain and Perrier [9] showed how to incorporate the well-known knowledge about the dynamics of biochemical processes in monitoring and control algorithms. Such methods are shown to be capable of dealing with process uncertainty by introducing an adaptation scheme.

### 2.2
### Optimizing control approaches

Optimizing control deals with the problem of changing the operating conditions of a continuous biochemical process to bring it to its optimum. In the earlier investigations carried out in this area, the optimization was based on an off-line identified mathematical model or on adhoc experimental procedures. However, the reliability of such models is questionable since process conditions vary continuously due to culture ageing, spontaneous mutations, wall growth and external disturbances. In order to overcome the aforementioned difficulties, another version of optimizing control was proposed where a linear dynamic on-line identified model is adaptively determined and its steady-state version is used to calculate the optimum. Rolf and Lim [10, 11] applied this method to optimize the volumetric productivity of baker's yeast fermentation in a chemostat through selection of optimal dilution rate. Harmon and coworkers [12] also illustrated these features by optimizing the production of biomass in a continuous fermenter. Hamer and Richenberg [13] employed this algorithm for on-line optimizing control of a packed bed immobilized cell bioreactor. Semones and Lim [14] carried out simulation studies and also implemented the algorithm to maximize steady-state cellular productivity of a continuous culture of baker's yeast by manipulating both temperature and dilution rate. A different approach of on-line optimizing control, in which nonlinear model identification and its dynamic optimization are carried out by decomposing the problem into two phases, was applied for optimizing biomass productivity in a chemostat and penicillin productivity in an immobilized cell fluidized bed reactor [15]. The first phase of this approach consists of identifying the unknown parameters and unmeasured disturbances entering the process by using all available process measurements. In the second phase, this identified nonlinear model is used to determine the optimal operating strategy. The two phases are periodically repeated in tandem to follow continually changing input disturbances and process parameters. In spite of the good performance of the two-phase approach, it has not found wide applicability since the two phases require solution of nonlinear static and nonlinear dynamic optimization at the end of every few sampling intervals, which is computationally intensive.

An alternative approach has been presented [16] for optimizing the steady-state productivity of a class of continuous fermentation processes described by an unstructured model. A simple substrate feeding rate control mechanism is developed for a prefixed dilution rate without the knowledge of certain process parameters, which was shown to be robust against measurement noise and process parameter variations. The presence of an extremum in the output and attendant change in the sign of the steady state gain poses a difficulty to a large number of nonlinear control strategies. A nonlinear control strategy that appears promising for handling such a singular situation is a nonlinear model predictive control (NMPC) strategy which uses a geometrically exact prediction model in the neighbourhood of the optimum point [17]. An on-line optimizing control scheme is proposed [18] for controlling a continuous fermenter based on this NMPC strategy. A nonlinear Laguerre model, whose parameters are estimated on-line, is used for tracking of the operating point, and the control at the operating point is achieved using an adaptive nonlinear predictive control strategy that uses the nonlinear Laguerre model for prediction.

A dual-mode adaptive control scheme is recently introduced and applied to a biochemical reactor, where in the first mode, the controller is designed to achieve stability and performance in the neighbourhood of the normal operating point, whereas in the second mode, the controller is designed to act as a safety jacket to bring the system from outside the normal regime to within the neighbourhood of the normal operating point [19]. Arnold et al. [20] presented a dynamic mechanistic model for a continuous extractive fermentation process, and also demonstrated its application for dynamic optimization of process start-up and changeover operation.

## 2.3 Linearization-based approaches

Nonlinear controllers based on exact linearization were also designed for continuous fermenters [21]. This approach is based on direct productivity control unlike earlier approaches where the productivity was indirectly controlled through cell, product or substrate regulation at specified values. State-space linearization and input-output linearizing control were explored using dilution rate or feed substrate concentration as manipulated variables to control the productivity at the optimum steady-state determined off-line a priori. The problems associated with each strategy are discussed and a modified input-output linearization approach, based on holding the substrate concentration constant near the optimum, was proposed and shown to result in satisfactory control. However, there are certain inherent problems associated with this control scheme. In the presence of unmeasured disturbances and unknown process changes, the optimum steady-state might change and can even become infeasible. Furthermore, in order to retain the feed substrate concentration near the optimum, the optimum point should be exactly identified and incorporated into the control strategy, which can cause problems in the face of process disturbances that influence the optimum operating point.

Recently, Rangiah and Hu [22] presented a Robust Internal Model Control (RIMC) strategy using linear and nonlinear models for continuous fermenter control where a complementary loop is found to improve the performance and robustness properties. The performance of nonlinear IMC was found to be comparable to that of linear RIMC and the nonlinear RIMC was shown to perform better than both these controllers. Ramseier et al. [23] presented a nonlinear adaptive control approach for fermentation control. Generic Model Control (GMC) was modified to be applicable to baker's yeast fermentation and a simple adaptive scheme was employed to update the parameters of a nonlinear model. Improvement in performance was achieved in course of simulations for multi-input multi-output GMC (cell concentration and product concentration using substrate feed concentration and dilution rate, respectively) and bench scale yeast fermentation experiments for single-input single-output GMC (cell mass concentration using dilution rate).

An adaptive version of linearizing control was proposed and applied to control hydrogen concentration in an anaerobic digestion process using dilution rate as the manipulated variable [24]. Dochain [25] proposed two algorithms for adaptive linearizing control of nonminimum phase bioprocesses, namely a dynamic feedback controller which is a continuous-time extension of generalized minimum variance control to bioreactors, and another design based on a minimum phase reduced order dynamic model of the process. The latter algorithm was shown to exhibit better performance in simulation studies using simple microbial growth process and an anaerobic digestion process. Developments in synthesis of SISO and MIMO adaptive linearizing algorithms for bioreactors were presented and illustrated through application to baker's yeast fermentation process [26]. An adaptive algorithm was proposed which enforces a desired and preset second-order convergence dynamics and provides the user with the choice of two simple and intuitive tuning parameters.

## 2.4 Habituating control

In order to overcome the problems associated with control of continuous fermenters using a single manipulated variable near the optimum operating point, habituating control strategy has been recently proposed [27]. This approach is based on using more than one manipulated inputs to control a single output variable. The motivation for this approach is based on human system where the baroreceptor reflexes are responsible for short term regulation of arterial blood pressure. Parasympathetic and sympathetic nervous systems act as the controllers to maintain the blood pressure at the desired level using cardiac output and the vascular resistance as the manipulated variables, respectively. Since sustained variations in cardiac output are physiologically more expensive as compared to the long-term variations in vascular resistance, the former mechanism is used preferentially during transient conditions (secondary input), whereas the latter is responsible for steady state control (primary input). Analogously, in the fermenter control problem, the primary control law is derived based on input-output linearization, whereas the secondary control law is based on minimizing the magnitude of deviation of both the input variables from steady-state values. This approach was employed to maintain the substrate concentration constant using dilution rate and dilution rate times the substrate feed concentration as manipulated variables and better performance is achieved compared to the use of a single manipulated variable.

## 3
## Batch fermenters

In batch fermentation, there are four phases of cell growth: induction, growth, stationary, and death phases. In the induction phase, the cells begin to adapt to their new environment and minimal reproduction occurs. Most of the cell growth occurs in the growth phase, when cells are dividing at the maximum rate. The cell growth is proportional to the cell concentration in this phase. In the stationary phase, the cell growth rate is virtually zero due to depletion of nutrients (organic feed) or crowding of cells. Finally, in the death phase, the cells begin to die and the growth rate becomes negative as a result of lack of nutrients or the presence of poisonous byproducts from the reaction.

Dynamic models of batch as well as fed-batch operations of industrial yeast fermenters have recently been reported [28]. You and Nikolaou [29] proposed the use of recurrent neural networks for dynamic process modeling and also employed a batch biochemical system modeling to illustrate their approach. Baughman and Liu [30] developed a time-dependent network for predictive modeling of batch fermentation using Saccharomyces cerevisae by linking three different types of neural networks. An autoassociative backpropagation network was used for data compression and filtering of the continuous cell concentration signal. A radial basis function classification network was then employed to identify the four phases of the fermentation process. The future cell concentrations were predicted using a recurrent time-dependent network attached to the growth phase output signal from the classification network. The overall network architecture is presented in Fig. 1. The network was shown to perform well in forecasting future cell concentrations over a wide range of operating conditions including temperature (25 to 35 °C), pH (3.5 to 5.5), agitation rate (200 to 600 rpm), aeration rate (0.0 to 5.0 $Nm^3/hr$), and glucose concentration (0 to 100 g/litre).

Simple continuous and discrete-time estimators which allow on-line estimation of the kinetics based on the measurements of concentrations of components in the bioreactors are proposed by Farza et al. [31, 32] and applied to a few simulations and a real-life batch experiment of lactic acid production. Cardello and San [33] presented a controller design approach for batch bioreactors where a combination of gain-scheduling PID controller with feed-forward-feedback control yielded best performance to control dissolved oxygen using oxygen uptake rate as the manipulated variable.

Shimizu [34] reviewed the current progress in bioprocess systems engineering, where it was suggested that for batch and fed-batch type of cultivation, the problem to be solved may be the on-line optimizing control problem. A hierarchical control structure (Fig. 2) can be considered, where the task of the upper layer is to recognise or learn the dynamics of the whole stage of cultivations, and to find the optimal trajectory using tools such as artificial neural networks. The task of the lower layer is to track the optimal trajectory. Predictive control strategy can be employed incorporating a modified optimal trajectory based on the current state information.

## 4
## Fed-batch fermenters

The most popular mode of operation of bioreactors has been the fed-batch mode where the substrate is slowly fed to the reactor but no product is drawn until the end. A fed-batch culture has the advantage of avoiding substrate overfeeding which can inhibit the growth of micro-organisms. The fact that no substance is withdrawn from the reactor, helps the process to work in good sterilized conditions [35]. For these reasons, this mode of production is often preferred to batch and continuous modes in many processes. On the other hand, from the control engineer's viewpoint, it is the fed-batch processes which present the greatest challenge: the process variables are difficult to measure, the "quality" of the product is difficult to define yet very important, the process model usually contains strongly time-varying parameters, etc. But above all, the challenge arises mainly because the optimization of the feedrate is a dynamical problem [2]. Efforts were concentrated on both modeling and estimation as well as control of fed-batch fermentation systems.

Fed-batch fermentation processes have been classified into three types based on the form of the specific growth

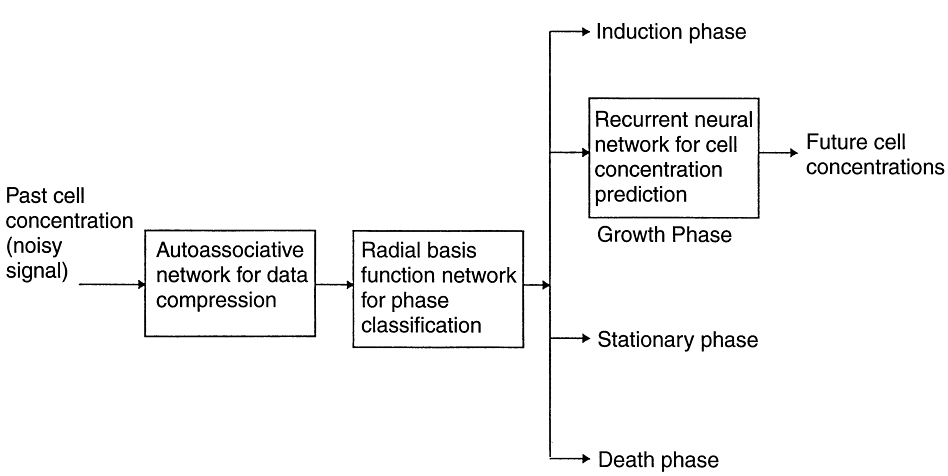


**Fig. 1.** Overall network architecture for prediction of future cell concentrations

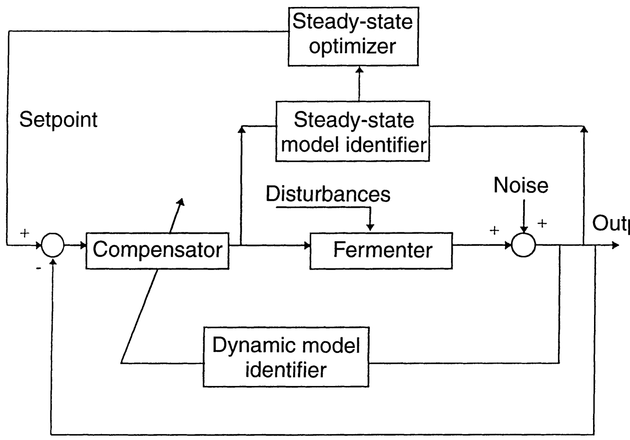


**Fig. 2.** Block diagram for on-line optimizing control

and product formation rates [36]. General dynamic model structure which encompasses batch, continuous as well as recycle microbial growth processes used as an aggregated model is modified into an age distribution model using average cell age [37]. Issues relating to model type and number of parameters are also discussed. Sensitivity function based parameter estimation techniques were employed for automatic parameter estimation of Michalis-Menton parameters. State estimation was carried out to estimate growth rate and biomass concentration and applied to an activated sludge waste water treatment process, a continuous fermentation process for single cell production and a batch fermentation process.

Stephanopoulos and San [38] suggested the use of additional state variables whose dynamics are expressed by differential equations obtained from adaptive estimation theories. Such an approach was used to obtain noise-free estimates of the state variables and culture parameters, online under both steady-state and dynamic conditions. San and Stephanopoulos [39] tested the on-line identification methodology and also studied the sensitivity of the estimation scheme with respect to respiratory quotient measurement. Sensitivity problems in using the respiratory quotient and heat evolution measurements have further been explored by Grosz et al. [40] and they suggested ATP balance to be an useful replacement to the sensitive measurements. Further studies by San and Stephanopoulos [41] indicated that pH measurements can be used for product estimation in cases of batch, fed-batch and continuous fermentation processes and illustrated the same by identification of continuous yeast fermentation to glucose.

An adaptive state estimator consisting of a linear Kalman filter whose parameters are simultaneously identified by a recursive prediction error algorithm was proposed for maintaining plasmid instability [42]. Two predictor structures were tried, namely a state-space model and the one using only output measurements, and their abilities and limitations were discussed.

The control approaches for fed-batch fermentation processes were classified [2] as physiological model and dynamic optimization approaches. While the former refers to the selection of a particular variable as the setpoint to be maintained constant on the basis of some theoretical reasoning without the use of mathematical models, the latter refers to maximizing/minimizing an objective function to find the optimal trajectory for setpoints to be tracked. Examples of physiological model control are substrate concentration control, specific growth rate control, ethanol control, respiration quotient control and quality control in a typical baker's yeast fermentation process. The second approach uses dynamic optimization involving iteration towards optimum by one of the four techniques based on Green's theorem, Pontryagin's maximum principle, Variational calculus, or Dynamic programming. Estimation of unmeasured states and environmental control for pH, temperature, etc. are also necessary to efficiently control a fed-batch fermentation process. Babila and Robinson [43] provided an extensive review on the optimal operation of a fed-batch process for monoclonal antibody production. They stated that the field of mammalian fed-batch culture control and optimization is still at its infancy compared to microbial fermentation control and optimization mainly because of lack of accurate mathematical models and reliable on-line biosensors.

### 4.1
### Optimal open-loop trajectory tracking approaches

Tartakovsky and coworkers [44] studied various control aspects of fed-batch auto-inductive fermentation process for metabolite production. An unstructured model of the process describing cell density, substrate and inducer concentrations was first derived and then used to determine the optimal open-loop control strategy composed of three stages, namely growth, inducer synthesis and product synthesis. Their results were validated by experimental verifications. Banga and co-workers [45, 46] proposed the use of two stochastic dynamic optimization algorithms for batch and fed-batch processes. These algorithms are based on a sequential control parametrization strategy: the original dynamic optimization problem is transformed into a constrained nonlinear programming (NLP) problem using parametrization of the control function and the constrained NLP is solved using stochastic algorithms such as Integrated Controlled Random Search for Dynamic Systems (ICRS/DS) and Adaptive Randomly Directed Search for Dynamic Systems (ARDS/DS). Open-loop optimal control strategies have been developed for penicillin production in a fed-batch reactor and dehydration of a bioproduct [45], and for protein production in a fed-batch bioreactor [46] which have resulted in better performance over previously reported studies. However, Banga et al. [45] have stressed the need for the development of tracking controllers for the implementation of the designed open-loop profiles as well as the need for on-line recalculation of the profiles in case of large disturbance. Recently, Wang and Shyu [47] developed an optimal feed policy for fed-batch fermentation of ethanol production by introducing additional inequality constraints in the optimization problem to assure optimal solution in a reality region. An updating rule of augmented Lagrange multipliers was introduced to handle inequality constraints so that Iterative Dynamic Programming could be used. The method was validated through experimental studies.

Nestaas and Wang [48] presented a structured model for penicillin fermentation which was then used in an open-loop control strategy to calculate the nutrient feed rate which maintains the cell mass concentration and growth rate at desired values, and in the event of changed growth conditions, a feedback correction term is introduced in the open-loop control law to adjust to the changed conditions and better results were obtained. Another contribution in tracking the open-loop optimal trajectory has been made by Gudi et al. [49] with the help of an adaptive multirate estimation and control strategy for the control of nutrient levels in a fed-batch fermentation process using both on-line and off-line measurements. The nutrient levels are estimated with the help of frequent online measurements of carbon dioxide evolution rate and off-line, infrequent and delayed measurements of biomass and substrate concentrations. The estimation algorithm is coupled with a nonlinear control law designed to track pre-specified optimal nutrient trajectories for simulation of fed-batch fermentation involving Streptomyces specie.

During the last decade, numerous laboratory studies have shown that improved production is obtained with feeding profiles that are calculated on-line in a feedback loop [50], unlike previous studies which used precalculated profiles. A novel feeding strategy, referred to as the modified linear feeding strategy (MLFS) is shown [51] to overcome the disadvantages of the exponential feedback strategy and the linear feeding strategy, resulting in a fast response in substrate concentration over a large range of initial conditions. The main feature of the MLFS is that it effectively decomposes the closed-loop system into fast and slow subsystems. The approach is illustrated as adaptive and nonadaptive cases through simulation studies.

## 4.2
## Singular control approaches

The most commonly encountered control policy in fed-batch fermenters is the singular control approach. San and Stephanopoulos [52] provided conditions along the singular arc and also for the final arc as part of the optimal feed-rate policy using maximum principle for fed-batch fermenters which follow Monod-type kinetics. General characteristics of optimal feed rate profiles for fed-batch fermentation processes were deduced by analysing singular controls and singular arcs [37]. Based on such characteristics, efficient computation algorithms have been developed and applied to simulations of penicillin fermentation and bacterial cell mass production [53].

For fed-batch fermenters with substrate inhibited kinetics, Cazzador [54] presented an approach to generate optimal feed rate policy using Green's theorem for maximization of biomass production and also accounting for time. In the presence of substrate and product inhibition kinetics, Hong [55] derived an optimal feeding policy analytically in terms of substrate and product concentrations and liquid volume by using Kelly's transformation to determine the conjunction point between nonsingular and singular arcs. Switching hypersurface and singular feed rate were expressed in terms of the state variables for online optimization in fed-batch fermenters and illustrated through application to Lysine fermentation and alchohol fermentation. Further, it was shown that accurate kinetic constants are indispensable for an optimization study. For the same fermentation system, Chen and Hwang [56] proposed an optimal on-off control solution which was derived for a general process described by differential algebraic equations. A unified algorithm was derived for computing the gradients of the cost function and constraints, which facilitates the solution of parameter selection problem resulting from on-off control parametrization by gradient-based optimization methods. They claimed to have obtained better results than those reported by Hong [55]. Renfro et al. [57] proposed simultaneous optimization and solution procedure for systems described by differential/algebraic systems using piecewise constant functions for independent variables that combines technologies of quasi-Newton optimization algorithms and global spline collocation, and applied it to batch reactor control problems. Cuthrell and Biegler [58] proposed an alternative simultaneous optimization and solution strategy based on SQP using orthogonal collocation on finite elements to discretize the differential equations, and Lagrange polynomials to construct approximations to continuous profiles and applied it to the fed-batch fermentation problem. The results obtained are reported to be better than the analytical solutions for biosynthesis of penicillin.

Gee and Ramirez [59] applied optimal temperature control by simulation as well as through experiments for batch beer fermentation. The objective functional was defined as maximizing the final ethanol concentration within minimum batch time and Pontryagin's maximum principle was used to determine the optimal control trajectory. An iterative algorithm was presented to compute switching times between bang-bang control and operation along a singular arc corresponding to maximum temperature constraint. However, they suggested that a more comprehensive model incorporating the effect of addition of various nutrients or oxygen at certain times can provide better control. Optimal state and parameter estimation techniques for such a comprehensive model of batch beer fermentation were presented by Ramirez [60] where coupling of sequential parameter estimating with Kalman filter state estimation was shown to be capable of estimating the entire state of the process even when some of the model parameters were uncertain. An optimal regulatory control law based on a combination of feed forward control action to follow predetermined setpoint trajectories augmented with estimates of provincial state variables with PI regulation to minimize setpoint deviation was proposed for regulation along singular arcs of glucose level that define the optimal policy in fed-batch reactors [61]. The optimal control policy for maximization of secreted protein in a fed-batch reactor was found to consist of multiple singular arcs and an iterative strategy was developed to find the optimal transitions between such arcs on the basis of minimum principle of Pontryagin [62]. Lee and Ramirez [63] derived singular control solutions for maximizing production of induced foreign protein by recombinant bacteria using nutrient and inducer feeding rates as control variables and compared the performance with bang-bang control solution and concluded that for the example considered, best bang-bang control is nearly equivalent to the optimal singular control.

### 4.3 Other optimal control approaches

In view of the problems associated with implementing singular control approaches, there have been certain attempts to avoid singular control. Menawat et al. [64] proposed a numerical technique embedding the singular control problem of maximizing production of baker's yeast in a fed-batch reactor in a sequence of nonsingular problems that converge to singular one in a limit as the ratio between the two time-scales ($\varepsilon$) tends to infinity. Yet another approach was proposed by San and Stephanopoulos [65] where the feed concentration is employed as a control variable in a solution that first determines the optimal reactor substrate concentration and subsequently solves for the feed concentration profile that results in the optimal substrate concentration profile. This approach was shown to result in better productivity for fed-batch penicillin production over other strategies. Further, Modak and Lim [66] proposed an approach based on defining a new set of variables and the use of culture volume as the control variable and solving the optimization problem numerically by steepest ascent or conjugate gradient methods. Another approach [67] is based on defining transformed control variables as substrate concentration in the fermenter, feed substrate concentration and mass flow rate of the substrate as against the use of substrate feed rate as the control variable. The conditions of optimality were demonstrated in each case and also conditions for equivalence of the four strategies established.

Luus and Rosen [68] proposed an iterative dynamic programming algorithm using penalty functions to handle final-state constraints. This is based on employing accessible grid points and region contraction. The proposed algorithm was used to find the optimal feed-rate policy for maximization of secreted protein in a fed-batch bioreactor reported by Park and Ramirez [62], and was shown to result in a better profit function when the batch time was increased by a small quantity. The same approach is applied for ethanol production [69] and 4% improvement was reported over the results obtained by Chen and Hwang [56]. Iterative dynamic programming (IDP) was used together with suitable penalty functions of absolute error values and also with move suppression factors for control action minimization for biosynthesis of penicillin in a fed-batch reactor [70]. This formulation is based on control parametrization proposed for solution of fixed terminal time optimal control problems subject to general constraints [71].

Chen et al. [35] stated three reasons for the use of advanced control strategies in fed-batch yeast production, namely the conflict between yield and productivity, enhancement of inhibitory substances due to the ethanol concentration, and reproducibility of cultivations. Five control strategies normally employed to combat these problems are respiratory quotient control, glucose concentration control at a low level, tracking an exponential profile for the amount of biomass, overall specific growth rate control, and ethanol concentration control or to track its profile. Of these strategies, Chen et al. [35] reported that ethanol concentration control is the better alternative since it corresponds to a good trade-off between yield and productivity. To improve the performance over PID controllers, model reference adaptive control [72] and self-tuning controllers were employed [73]. However, it is more interesting to exploit the nonlinear structure of fed-batch systems and therefore adaptive nonlinear regulation was proposed. This control problem has been solved in four steps [35]: design of biomass estimator independent of the kinetics; model reduction based on qualitative information about the system; design of model-reference feed-back linearizing control law based on the reduced order model; and design of parameter adaptation law. The proposed algorithm was tested on an industrial application. In an earlier study, Staniskis and Levisauskas [74] also proposed an adaptive control algorithm for a fed-batch culture to maximize the output of the product. Dynamic model based on batch and semi-batch experiments was derived, transformed into a form suitable for estimation of states and model parameters, and employed in the optimal control law which was divided into three parts. The first and last phases involved no feeding whereas in the second phase, substrate was fed at the rate calculated by the singular control law.



An input-output linearizing controller was developed [75] to follow the open-loop optimal setpoint of substrate concentration using feed rate as the manipulated variable with the help of a nonlinear state observer for estimation of unmeasured state variables. Their results illustrate the improvement in performance over open-loop control implementation.

## 5 Neural Network based approaches

There has been a considerable interest recently in the possibilities offered by Neural Networks (NN) in process modeling as well as control of chemical and biochemical processes. The inherent capability of the NNs to handle general nonlinear relationships has led to their extensive use in different applications. Thibault et al. [76] introduced the use of NN computational algorithms for dynamic modeling of bioprocesses. The performance of the network is compared to that of an extended Kalman filter (EKF) and was shown to exhibit comparable performance in case of a continuous stirred tank fermenter. Massimo et al. [77] also investigated the construction of NN-based biomass and penicillin estimators for use in industrial fermentations. Their results demonstrated how an artificial neural network of modest scale could capture complex bioprocess dynamics. Breusegem et al. [78] used neural networks for on-line prediction of fermentation variables when kinetic changes appear during the course of fermentation. They proposed an adaptive algorithm in which sliding window learning schemes are used.

Bhat and McAvoy [79] proposed the use of NNs for dynamic modeling and later to be used as a neural model together with optimization for control, or as identification of the inverse model which is used as a direct inverse controller. Several NN based control strategies such as

Nonlinear Internal Model Control-NIMC [80], Nonlinear Model Predictive Control-NMPC [81, 82] were proposed for chemical process control. A neural type of nonlinear autoregressive with exogeneous input (NARX) model was used [83] for neural based predictive control of simulated microalgae fermentation process and a comparison with predictive control based on adaptive polynomial NARX model illustrated a satisfactory control behaviour. Another form of neural predictive control has been presented [84] using two different types of neural networks – feedforward and radial basis function nets where a new method is proposed to train the parameters of the net. The approach is tested on a bioreactor and its robustness compared with other advanced control algorithms. A novel architecture of controller based on affine radial basis function network approximation has recently been presented [85] as an adaptive control algorithm so that the inversion with respect to control vector can be carried out by fast vector computations. The approach has been applied to a benchmark bioreactor problem with no apriori knowledge of its dynamics.

Most of the above mentioned approaches are based on Feedforward Neural Networks (FNN). However, from the control point of view, a very important issue to be considered is the feedback issue. Attempts have been made to incorporate this component by way of development of Recurrent Neural Network (RNN)-based control approaches. You and Nikolaou [29] studied a method of nonlinear static and dynamic process modeling via RNNs. The two salient features that distinguish RNNs from FNNs are their node characteristics and their topology. The node characteristics in RNNs involve nonlinear dynamic functions (ordinary differential equations) while FNNs have only static nonlinear characteristics. The topology in RNNs consists of both feedforward and feedback connections whereas in FNNs there are only the feedforward paths. You and Nikolaou [29] further showed that the modeling capabilities of RNNs and FNNs are comparable, but the training of RNNs takes a longer time. They carried out simulation studies using continuous and batch, SISO and MIMO systems including a biochemical batch system. Karim and Rivera [86] used FNNs and RNNs as unmeasurable state estimators to predict biomass, ethanol and glucose concentrations with temperature, redox potential, percentage carbondioxide and optical density as inputs. In general, it was found that both types of NNs offer comparable abilities to recall, whereas the recurrent networks performed better in generalization studies. An exact linearization controller based on RNNs was devised and applied to a CSTR which shows the applicability of RNNs in control [87]. Real-time recurrent learning algorithm based neural network architecture was recently proposed where a combined network cluster consisting of the control network and the model network is constructed [88]. The proposed algorithm was tested on a bioreactor model which is to be used as benchmark problem for neural controllers. Adaptive pH control was carried out in batch fermentation [89] using a 4-4-1 recurrent backpropagation neural network using pH setpoint as its input node. A moving window of training data was used for effective online learning in the first phase of the two-phase model operation, whereas in the second phase, the model was used to predict the pump flow rate at the next sampling instant and good results were obtained.

## 6 Hybrid neural network based approaches

There are a few drawbacks associated with the use of NNs-no general procedure of NN architecture selection, long training time, large amount of training data, and poor capacity for extrapolation. Further, Ponton and Klemes [90] argued that a model incorporating any engineering or scientific knowledge will perform much better than NN. They suggested certain non-neural network models with lesser number of parameters and showed with a few typical examples that the performance is much better than with NNs. They further emphasized that practically any model with a physical basis, even if highly approximated, is likely to be better than any arbitrary functions for representing a physical system.

This observation leads one to an alternative approach which is slightly different from the "black box" NN approach, namely the hybrid NN approach. Psichogios and Ungar [91] developed a hybrid NN-first principles modeling scheme and used it to model a fed-batch bioreactor. In this approach, the first principles partial model specifies process variable interactions from physical considerations whereas the NN complements this model by estimating unmeasured process parameters so as to satisfy the first principles constraints. This form of hybrid NN (structured network) is useful for modeling processes where a partial model can be derived from simple physical considerations (mass and energy balances), but which also includes terms that are difficult or infeasible to model from first principles. In the case of fed-batch bioreactor, the specific growth rate term, in which the kinetics is imbedded, is modelled as the NN with the dynamic equations for cell mass and substrate concentrations representing the first principles model. The advantages of this approach are: (1) the hybrid model uses its internal structure to restrict the interactions among process variables to be consistent with physical models, (2) results in a more accurate generalization and extrapolation over standard networks and (3) requires less data for training. For process parameters which are rapidly time-varying and which are not easily described by a parameterized model, it was shown that hybrid NNs outperform other estimation methods such as EKF and nonlinear programming methods. The applicability of the hybrid model was further illustrated [92] by introduction of ANN into detailed simulations of complex biochemical processes. The mechanistic part of the model was composed of 44 variables and 145 parameters, where the NN was used to update 15 key parameters using reinforcement learning scheme. It was shown that the combined approach yielded better predictions than using either of the approaches independently.

Tholudur and Ramirez [93] classified the NN modeling into two categories, namely NN state modeling and NN parameter function modeling. In the former approach, the states of the process are estimated using information of previous states and inputs. This approach can be considered identical to the modeling approach using NNs only, such as FNNs or RNNs or RBFNs, mentioned previously. On the other hand, in the latter approach, which is the same as the hybrid approach, the nonlinear (unknown) functions which form a part of the balance equations are predicted by training NNs using information on states and state derivatives. This latter approach was used together with Iterative Dynamic Programming (IDP) in finding the optimal feed policy in two fed-batch fermenters. The number of hidden layer neurons is considered as a design parameter, and a simple method was suggested to select this parameter. A similar approach which uses a combination of NNs with a linear model was proposed by Su and McAvoy [94]. The concept is based on a Hammerstein modeling approach where a static nonlinear operator acts on the input, followed by a dynamic linear operator resulting in the output. Neural Network is used as the static nonlinear operator so that the nonlinearity which is associated to a large extent in the steady-state part is captured, and this is supplemented by a linear dynamic model since the transient data might not cover nonlinearity in most of the cases. Recently, the hybrid modelling approach has been compared with the conventional approach by De Azevedo et al. [95] by considering baker's yeast fermentation at laboratory scale as a case study. Three modelling approaches are described and compared-conventional mechanistic model, formulations based on different ANN topologies, and a hybrid mechanistic ANN structure. It was shown that the first two tests failed in the validation test of experimental test data, whereas the third structure was found to be a powerful tool for process modelling in biochemical engineering, particularly when limited theoretical knowledge of the process is available.

A different type of hybrid approach [96] derived by inversion of a multilayer feedforward network using Newton Raphson method by combination with a conventional feedback controller to suppress modelling errors is proposed and applied to a bioreactor simulation system to show its effectiveness. Back-propagation-through-time (BPTT) is a temporal extension of back propagation which allows a multilayer neural network to approximate an optimal state feed back control law, provided some prior knowledge of the process is available. A simplified version of this algorithm has been presented [97] which is less time-consuming and allows discovery of better control laws. The improvement is illustrated through a bioreactor control study. Another recent study [98] illustrated that to obtain an accurate model, one needs to use steady-state data in addition to the transient data for training the network, and it was also shown that a hybrid approach exhibits significantly better performance than the black box model in a bioreactor control problem.

A more sophisticated type of hybrid model was formulated for yeast production process [99] in which the structured hybrid NN previously mentioned is combined with a fuzzy expert system that serves to divide the whole process into typical situations, or for supervisory control where neither the mathematical process model nor the NN was sufficiently accurate. This approach was validated for state estimation and prediction with data from a pilot-scale fermenter. Ishida [100] proposed a policy-and-experience driven NN with a fixed architecture for control which can also be viewed as this type of hybrid approach.

In the hybrid NN approaches discussed so far, the network was restricted to the most common Back Propagation feedforward Network (BPN). Since radial basis function networks (RBFNs) are claimed to provide a better generalization over BPNs, Thompson and Kramer [101] proposed a hybrid model for predicting cell biomass and secondary metabolite in a fed-batch penicillin fermentation using prior knowledge and RBFNs. They compared the performance of the hybrid model to that of a pure BPN and a pure RBFN model and claimed to have improved the accuracy of prediction even in the presence of noisy measurements. The approach was also shown to require less data for parameter estimation, to enhance the generalization capabilities over pure networks, and to provide more reliable extrapolation.

A five-layered neural-fuzzy network was developed for controlling fed-batch cultivation of recombinant Escherichia Coli [102, 103]. The architecture of the network has an input layer consisting of deviations of pH and specific cell growth rate from their respective setpoints as two inputs, three hidden layers and an output layer. The three hidden layers represent the membership functions, measures of rule strength, and normalized measures of rule strength, respectively. The glucose feed rate was adjusted based on the compensation factor which is the output of the network, and the performance was shown to improve from 20 dry cell weight/litre under conventional control to 84 dry cell weight/litre under neural fuzzy control.

## 7
## Conclusions

The present study is aimed at reviewing the recent literature in fermenter control (continuous as well as batch and fed-batch) along with certain advancements in the related areas of research, in order to pave way for a better understanding and utilization of the available information towards reducing production costs, increasing the yield, and maintaining the desired product quality. Several neural network based control approaches with application to fermenter control have also been reviewed in this study.

Control of fermentation processes has been attracting more attention over the past few years due to their inherent difficulties such as nonlinearity, lack of accurate process models and reliable biosensors, and unpredictable variations in process parameters. In this study, an attempt is made to review the recent research developments in this area. The control objective in continuous fermenters is to maximize the amount of desired product produced per unit time, whereas in batch and fed-batch fermenters, the amount of desired product at the end of the batch is to be maximized; this situation has led to problems of different nature in the two cases.

In continuous fermentation processes, the earliest development was off-line estimation of optimal setpoint profiles and setpoint tracking using several alternative configurations. Later, simple nonlinear models were employed where the parameters with physical meaning were updated and adaptive control was implemented. Further, optimizing control approaches with linear dynamic model identification and periodic computation of the steady-state optimum based on the updated model were used. These approaches were further improved by using two-phase approach of nonlinear dynamic model identification and dynamic optimization over time periods covering a few sampling intervals. Optimizing control using MPC strategy based on on-line identified nonlinear Laguerre model is also proposed for bioreactors. Another type of dual-mode controller was proposed for continuous fermenter control, where in the first mode, the controller was designed to achieve stability and performance in the neighbourhood of the normal operating point, whereas in the second mode, the controller's task was to bring the process to the neighbourhood of the normal operating point. State-space and input-output linearization based approaches were also presented with several modifications to suit the specific fermenter control problem.

Batch fermenters have been controlled using gain-scheduling PID controllers, and also by employing artificial neural networks to find the setpoint trajectory which was then implemented through predictive control. Work on fed-batch fermenters has been widely reported in literature. The approaches employed have been the optimal open-loop trajectory tracking with or without feedback correction for adaptation to changed conditions. Singular control approaches were used to obtain the optimal feed-rate policy using Maximum principle, Green's theorem or other optimization methods. Some approaches were also presented as alternatives to singular control approach where the problems were either split into parts which were non-singular, or where new sets of variables were defined to avoid singularity problems. Finally, input-output linearizing controller was also proposed to implement optimal feed-policy for fed-batch fermenter control with the help of a nonlinear observer.

Different types of NNs including FNNs and RNNs have been used in different applications, namely for nonlinear state estimation, dynamic modeling and control. Several studies using these networks illustrated the comparable performance of FNNs and RNNs. This range clearly illustrates the usefulness of the newly emerging mathematical tool.

Another approach which holds considerable promise is the hybrid NN approach. These networks are used to augment the existing information about the physical model of the process. Therefore, this approach is especially suitable to fermentation processes where the general structure for the first principles model is well-established, but the kinetics involving microorganism is still difficult to model. Hybrid NNs were shown to outperform conventional estimation methods such as EKF and NLP. RBFNs as well as BPN with sigmoidal activation functions were employed in several case studies reported in literature for fermenter control. A combination of fuzzy expert system and neural networks was proposed and validated with the help of pilot-plant fermenter studies.

In conclusion, this study has been an attempt to review different methods of controlling batch, fed-batch and continuous fermenters as reported in literature. Several tools were explored to capture the inherent nonlinear and time-varying characteristics of fermentation processes. Although adaptive control has been successfully implemented at the regulatory level, little research has been carried out to develop a comprehensive approach where the supervisory as well as regulatory levels operate adaptively to follow the time-varying characteristics of bioprocesses. Therefore, there is scope for further research in the area of simultaneous optimum setpoint generation and implementation in continuous fermenters, and in on-line generation of optimal setpoint profiles and their effective implementation in fed-batch fermenters with the help of powerful mathematical tools like neural networks.

## References


1. **Bogle, D.L.; Cockshott, A.R.; Bulmer, M.; Thornhill, N.; Gregory, M.; Dehghani, M.:** A process systems engineering view of biochemical process operations. Comput. Chem. Eng. 20(6/7) (1996) 943–949
2. **Johnson, A.:** The control of fed-batch fermentation processes – a survey, Automatica 23(6) (1987) 691–705
3. **Modak, J.M.; Lim, H.C.:** Optimal mode of operation of bioreactor for fermentation processes. Chem. Eng. Sci. 47(15/16) (1992) 3869–3884
4. **Menawat, A.S.; Balachander, J.:** Alternate control structures for chemostat. A.I.Ch.E. J. 37(2) (1991) 302–306
5. **Zhao, Y.; Skogestad, S.:** Comparison of various control configurations for continuous bioreactors. Ind. Eng. Chem. Res. 36(3) (1997) 697–705
6. **Dochain, D.; Bastin, G.:** Adaptive identification and control algorithms for nonlinear bacterial growth systems. Automatica 20(5) (1984) 621–634
7. **Renard, P.; Dochain, D.; Bastin, G.; Naveau, H.; Nyns, E.H.:** Adaptive control of anaerobic digestion processes – a pilot scale application. Biotechnol. Bioeng. 31(3) (1988) 287–294
8. **Suarez-Cortez, R.; Alvarez-Gallegos, J.; Gonzales-Mora, E.:** Sliding controller design for a nonlinear fermentation system. Biotechnol. Bioeng. 33(4) (1989) 377–385
9. **Dochain, D.; Perrier, M.:** Dynamical modelling, analysis, monitoring and control design for nonlinear bioprocesses. Adv. Biochem. Eng. Biotechnol. 56 (1997) 147–197
10. **Rolf, M.J.; Lim, H.C.:** Adaptive on-line optimization for chemical bioreactors. Chem. Eng. Commun. 29 (1984) 229–255
11. **Rolf, M.J.; Lim, H.C.:** Experimental adaptive on-line optimization of cellular productivity of a continuous baker's yeast culture. Biotechnol. Bioeng. 27(8) (1985) 1236–1245
12. **Harmon, J.; Svoronos, S.A.; Lyberatos, G.:** Adaptive steady-state optimization of biomass productivity in continuous fermentors. Biotechnol. Bioeng. 30(3) (1987) 335–344
13. **Hamer, J.W.; Richenberg, C.B.:** On-line optimizing control of a packed-bed immobilized cell reactor. A.I.Ch.E. J. 34(4) (1988) 626–632
14. **Semones, G.B.; Lim, H.C.:** Experimental multivariable adaptive optimization of the steady-state cellular productivity of a continuous baker's yeast culture. Biotechnol. Bioeng. 33(1) (1989) 16–25
15. **Rani, K.Y.; Gangiah, K.:** Adaptive on-line optimizing control of bioreactors: a two-phase approach. Indian J. Technol. 30(1) (1992) 1–6
16. **Nguang, S.K.; Chen, X.D.:** Simple substrate feeding rate control mechanism for optimizing the steady state productivity of a class of continuous fermentation processes. Biotechnol. Prog. 13(2) (1997) 200–204
17. **Patwardhan, S.C.; Madhavan, K.P.:** Nonlinear predictive control using approximate second order perturbation model. Ind. Eng. Chem. Res. 32 (1993) 334–344
18. **Saha, P.K.; Patwardhan, S.C.; Rao, V.S.R.:** Maximizing productivity of a continuous fermenter using nonlinear

adaptive optimizing control. Bioprocess Eng. (1998) (in press)
19. **Allgower, F.; Ogunnaike, B.A.:** Dual-mode adaptive control of nonlinear processes. Comput. Chem. Eng. 21 (Suppl.) (1997) S155–S160
20. **Arnold, S.G.; Mclellan, P.J.; Daugulis, A.J.:** Dynamic modeling and performance optimization of an extractive fermentation. Can. J. Chem. Eng. 74(3) (1996) 385–393
21. **Henson, M.A.; Seborg, D.E.:** Nonlinear control strategies for continuous fermenters. Chem. Eng. Sci., 47(4) (1992) 821–835
22. **Rangiah, G.P.; Hu, Q.:** Robust Control of a Continuous Fermenter. Proceedings of the Indian Institute of Chemical Engineers Golden Jubilee Congress, New Delhi (Dec. 15–18, 1997) 1059–1067
23. **Ramseier, M.; Agrawal, P.; Mellichamp, D.A.:** Nonlinear adaptive control of fermentation processes utilizing a priori knowledge. Proc. ACC (1991) 1305–1310
24. **Dochain, D.; Perrier, M.; Pauss, A.:** Adaptive control of the hydrogen concentration in anaerobic digestion. Ind. Eng. Chem. Res. 30(1) (1991) 129–136
25. **Dochain, D.:** Adaptive control algorithms for nonminimum phase nonlinear bioreactors. Comput. Chem. Eng. 16(5) (1992) 449–462
26. **Ferriera, E.C.; De Azevedo, S.F.:** Adaptive linearizing control of bioreactors. UK ACC International Conf. Control'96. 2 (1996) 1184–1189
27. **Mclain, R.B.; Kurtz, M.J.; Henson, M.A.:** Habituating control for nonsquare nonlinear processes. Ind. Eng. Chem. Res. 35(11) (1996) 4067–4077
28. **Pertev, C.; Turker, M.; Berber, R.:** Dynamic modeling, sensitivity analysis and parameter estimation of industrial yeast fermenters. Comput. Chem. Eng. 21 (Suppl.) (1997) S739–S744
29. **You, Y.; Nikolaou, M.:** Dynamic process modeling with recurrent neural networks. A.I.Ch.E. J. 39(10) (1993) 1654–1667
30. **Baughman, D.R.; Liu, Y.A.:** Neural networks in bioprocessing and chemical engineering, Academic press (1995) 235–269
31. **Farza, M.; Othman, S.; Hammouri, H.; Bistin J.:** A nonlinear approach for the on-line estimation of kinetic rates in bioreactors: Application to a lactic acid production process. Bioprocess Eng. 17(3) (1997) 143–150
32. **Farza, M.; Othman, S.; Hammouri, H.; Fick, M.:** Discrete time nonlinear observer based estimators for the on-line estimation of kinetic rates in bioreactors. Bioprocess Eng. 17(4) (1997) 247–255
33. **Cardello, R.J.; San, K.Y.:** The design of controllers for batch bioreactors. Biotechnol. Bioeng. 32(8) (1988) 519–526
34. **Shimizu, K.:** A tutorial review on bioprocess systems engineering. Comput. Chem. Eng. 20(6/7) (1996) 915–941
35. **Chen, L.; Bastin, G.; Breusegem, V.V.:** A case study of adaptive nonlinear regulation of fed-batch biological reactors. Automatica 31(1) (1995) 55–65
36. **Modak, J.M.; Lim, H.C.; Tayeb, Y.J.:** General characteristics of optimal feed rate profiles for various fed-batch fermentation processes. Biotechnol. Bioeng. 28(9) (1986) 1396–1407
37. **Holmberg, A.; Ranta, J.:** Procedures for parameter and state estimation of microbial growth process models. Automatica 18(2) (1982) 181–193
38. **Stephanopoulos, G.; San, K.Y.:** Studies on on-line bioreactor identification. I. Theory. Biotechnol. Bioeng. 26(10) (1984) 1176–1188
39. **San, K.Y.; Stephanopoulos, G.:** Studies on on-line bioreactor identification. II. Numerical and experimental results. Biotechnol. Bioeng. 26(10) (1984) 1189–1197
40. **Grosz, R.; Stephanopoulos, G.; San, K.Y.:** Studies on on-line bioreactor identification. III. Sensitivity problems with respiratory and heat evolution measurements. Biotechnol. Bioeng. 26(10) (1984) 1198–1208
41. **San, K.Y.; Stephanopoulos, G.:** Studies on on-line bioreactor identification. IV. Utilization of pH measurements for product estimation. Biotechnol. Bioeng. 26(10) (1984) 1209–1218
42. **Chattaway, T.; Stephanopoulos, G.:** Adaptive estimation of bioreactors: monitoring plasmid instability. Chem. Eng. Sci. 44(1) (1989) 41–48
43. **Babila, T.A.; Robinson, D.K.:** In pursuit of the optimal fed-batch process for monoclonal antibody production. Biotechnol. Prog. 11(1) (1995) 1–13
44. **Tartakovsky, B.; Ulitzur, S.; Sheintuch, M.:** Optimal control of fed-batch fermentation with auto-induction of metabolite production. Biotechnol. Prog. 11(1) (1995) 80–87
45. **Banga, J.R.; Alonso, A.A.; Singh, R.P.:** Stochastic dynamic optimization of batch and semicontinuous bioprocesses. Biotechnol. Prog. 13(3) (1997) 326–335
46. **Carrasco, E.F.; Banga, J.R.:** Dynamic optimization of batch reactors using adaptive stochastic algorithms. Ind. Eng. Chem. Res. 36(6) (1997) 2252–2261
47. **Wang, F.S.; Shyu, C.S.:** Optimal feed policy for fed-batch fermentation of ethanol production by Zymomous mobilis. Bioprocess Eng. 17(2) (1997) 63–68
48. **Nestaas, E.; Wang, D.I.C.:** Computer control of the penicillin fermentation using the filtration probe in conjunction with a structured model. Biotechnol. Bioeng. 25(3) (1983) 781–796
49. **Gudi, R.D.; Shah, S.L.; Gray, M.R.; Yagneswaran, P.K.:** Adaptive multirate estimation and control of nutrient levels in a fed-batch fermentation using off-line and on-line measurements. Can. J. Chem. Eng. 75(3) (1997) 562–573
50 **Boskovic, J.D.:** Novel feeding strategy for adaptive control of bioreactor processes. Journal of Process Control. 7(3) (1997) 209–217
51. **Modak, J.M.; Lim, H.C.:** Feedback optimization of fed-batch fermentation. Biotechnol. Bioeng. 30(9) (1987) 528–540
52. **San, K.Y.; Stephanopoulos, G.:** A note on the optimality criteria for maximum biomass production in a fed-batch fermenter. Biotechnol. Bioeng. 26(10) (1984) 1261–1264
53. **Lim, H.C.; Tayeb, Y.J.; Modak, J.M.; Bonte, P.:** Computational algorithms for optimal feed rates for a class of fed-batch fermentation: Numerical results for penicillin and cell mass production. Biotechnol. Bioeng. 28(9) (1986) 1408–1420
54. **Cazzador, L.:** On the optimal control of fed-batch reactors with substrate-inhibited kinetics. Biotechnol. Bioeng. 31(5) (1988) 670–674
55. **Hong, J.:** Optimal substrate feeding policy for a fed-batch fermentation with substrate and product inhibition kinetics. Biotechnol. Bioeng. 28(9) (1986) 1421–1431
56. **Chen, C.T.; Hwang, C.:** Optimal on-off control for fed-batch fermentation processes. Ind. Eng. Chem. Res. 29(9) (1990) 1869–1875
57. **Renfro, J.G.; Morshedi, A.M.; Asbjornsen, O.A.:** Simultaneous optimization and solution of systems described by differential/algebraic equations. Comput. Chem. Eng. 11(5) (1987) 503–517
58. **Cuthrell, J.E.; Biegler, L.T.:** Simultaneous optimization and solution methods for batch reactor control profiles. Comput. Chem. Eng. 13(1/2) (1989) 49–62
59. **Gee, D.A.; Ramirez, W.F.:** Optimal temperature control for batch beer fermentation. Biotechnol. Bioeng. 31(2) (1988) 224–234
60 **Ramirez, W.F.:** Optimal state and parameter estimation. an application to batch fermentation. Chem. Eng. Sci. 42(11) (1987) 2749–2756
61. **Park, S.; Ramirez, W.F.:** Optimal regulatory control of bioreactor nutrient concentration incorporating system identification. Chem. Eng. Sci. 45(12) (1990) 3467–3481

62. **Park, S.; Ramirez, W.F.:** Optimal production of secreted protein in fed-batch reactors. A.I.Ch.E.J. 34(9) (1988) 1550–1558
63. **Lee, J.; Ramirez, W.F.:** Optimal fed-batch control of induced foreign protein production by recombinant bacteria. A.I.Ch.E.J. 40(5) (1994) 899–907
64. **Menawat, A.; Mutharasan, R.; Coughanowr, R.:** Singular optimal control strategy for a fed-batch bioreactor: numerical approach. A.I.Ch.E.J. 33(5) (1987) 776–783
65. **San, K.Y.; Stephanopoulos, G.:** Optimization of fed-batch fermentation: a case of singular optimal control with state constraints. Biotechnol. Bioeng. 34(1) (1989) 72–78
66. **Modak, J.M.; Lim, H.C.:** Simple nonsingular control approach to fed-batch fermentation optimization. Biotechnol. Bioeng. 33(1) (1989) 11–15
67. **Modak, J.M.:** Choice of control variable for optimization of fed-batch fermentation. The Chem. Eng. J. 52(3) (1993) B59–B69
68. **Luus, R.; Rosen, O.:** Application of dynamic programming to final state constrained optimal control problems. Ind. Eng. Chem. Res. 30(7) (1991) 1525–1530
69. **Luus, R.:** Application of dynamic programming to differential algebraic process systems. Comput. Chem. Eng. 17(4) (1993) 373–377
70. **Dadebo, S.A.; Mcauley, K.B.:** Dynamic optimization of constrained chemical engineering problems using dynamic programming. Comput. Chem. Eng. 19(5) (1995) 513–525
71. **Goh, C.J.; Teo, K.L.:** Control parametrization: a unified approach to optimal control problems with general constraints. Automatica 24(1) (1988) 3–18
72. **Takamatsu, T.; Shioya, S.; Okada, Y.; Kanda, M.:** Profile control scheme in a baker's yeast fed-batch culture. Biotechnol. Bioeng. 27(12) (1985) 1675–1686
73. **Williams, D.; Yousefpour, P.; Wellington, E.M.H.:** On-line adaptive control of a fed-batch fermentation of saccharomyces cerevisiae. Biotechnol. Bioeng. 28(5) (1986) 631–645
74. **Staniskis, J.; Levisauskas, D.:** An adaptive control algorithm for fed-batch culture. Biotechnol. Bioeng. 26(5) (1984) 419–425
75. **Tartakovsky, B.; Dainson, B.E.; Lewin, D.R.; Sheintuch, M.:** Observer based nonlinear control of auto-inductive fed-batch fermentation process. The Chem. Eng. J. 61(1) (1996) 139–148
76. **Thibault, J.; Breusegem, V.V.; Cheruy, A.:** On-line prediction of fermentation variables using neural networks. Biotechnol. Bioeng. 36(12) (1990) 1041–1048
77. **Massimo, C.D.; Montague, G.A.; Willis, M.A.; Tham, M.T.; Morris, A.J.:** Towards improved penicillin fermentation via artificial neural networks. Comput. Chem. Eng. 16(4) (1992) 283–291
78. **Breusegem, V.V.; Thibault, J.; Cheruy, A.:** Adaptive neural models for on-line prediction in fermentation. Can. J. Chem. Eng. 69(2) (1991) 481–487
79. **Bhat, N.; McAvoy, T.J.:** Use of neural networks for dynamic modeling and control of chemical process systems. Comput. Chem. Eng. 14(4/5) (1990) 573–583
80. **Nahas, E.P.; Henson, M.A.; Seborg, D.E.:** Nonlinear internal model control strategy for neural network models. Comput. Chem. Eng. 16(12) (1992) 1039–1057
81. **Psichogios, D.; Ungar, L.H.:** Direct and indirect model based control using artificial neural networks. Ind. Eng. Chem. Res. 30(12) (1991) 2564–2573
82. **Song, J.J.; Park, S.:** Neural model predictive control for nonlinear chemical processes. J. Chem. Eng. Japan 26(4) (1993) 347–354
83. **Hong, T.; Zhang, J.; Morris, A.J.; Martin, B.; Karim, M.N.:** Neural based predictive control of a multivariable microalgae fermentation. Proc. IEEE International Conference on Systems, Man and Cybernetics, 1 (1996) 345–350
84. **Declercq, F.; DeKeyser, R.:** Neural model based predictive control of a simulated bioreactor. Symp. Control, Optimization and Supervision, CESA' 96 IMACS Multiconference, Computational Engineering in Systems Applications
85. **Gorinvesky, D.:** Sampled-data indirect adaptive control of bioreactor using affine radial basis function architecture. Transactions of the ASME, Journal of Dynamic Systems, Measurement and Control, 119(1) (1997) 94–97
86. **Karim, M.N.; Rivera, S.L.:** Comparison of feedforward and recurrent neural networks for bioprocess state estimation. Comput. Chem. Eng. 16 (1992) S369–S375
87. **Nikolaou, M.; Hanangadi, V.:** Control of nonlinear dynamical systems modeled by recurrent neural networks. A.I.Ch.E.J. 39(11) (1993) 1890–1894
88. **Chovan, T.; Catfolis, T.; Meert, K.:** Neural network architecture for process control based on RTRL algorithm. A.I.Ch.E.J. 42(2) (1996) 493–502
89. **Syu, M.J.; Chang, C.B.:** Neural network adaptive control of the penicillin acylase fermentation, paper presented at the IEE International Conference on Neural Networks, (1997)
90. **Ponton, J.W.; Klemes, J.:** Alternatives to neural networks for inferential measurement. Comput. Chem. Eng. 17(10) (1993) 991–1000
91. **Psichogios, D.; Ungar, L.H.:** A hybrid neural network-first principles approach to process modeling. A.I.Ch.E.J. 38(10) (1992) 1499–1511
92. **Fu, P.C.; Barford, J.P.:** A hybrid neural network-first principles approach for modeling of cell metabolism. Comput. Chem. Eng. 20(6/7) (1996) 951–958
93. **Tholudur, A.; Ramirez, W.F.:** Optimization of fed-batch bioreactors using neural network parameter function models. Biotechnol. Prog. 12(3) (1996) 302–309
94. **Su, H.T.; McAvoy, T.J.:** Integration of multilayer perceptron networks and linear dynamic models. Ind. Eng. Chem. Res. 32(9) (1993) 1927–1936
95. **De Azevedo, S.F.; Dahm, B.; Olivìera, F.R.:** Hybrid modelling of biochemical processes: A comparison with conventional approach. Comput. Chem. Eng. 21(S) (1997) S751–S756
96. **Bersini, H.; Gorrini, V.:** A simplification of the back propagation through time algorithm for optimal neurocontrol. IEEE Trans. Neural Networks. 8(2) (1997) 437–441
97. **Lee, J.W.; Oh, J.H.:** Inversion control of nonlinear systems with neural network modelling. IEEE Proc.-Control Theory and Applications, 144(5) (1997) 481–487
98. **Ayoma, A.; Venkatasubramianian, V.:** Internal model control framework using neural networks for modeling and control of a bioreactor. Engineering Applications of Artificial Intelligence 8(6) 689–701
99. **Schubert, J.; Simutis, R.; Dors, M.; Havlik, I.; Lubbert, A.:** Hybrid modeling of yeast production processes-combination of a priori knowledge on different levels of sophistication. Chem. Eng. Technol. 17(1) (1994) 10–20
100. **Ishida, M.:** Control by a policy-and-experience-driven neural network. J. Chem. Eng. Japan 25(1) (1992) 108–111
101. **Thompson, M.L.; Kramer, M.A.:** Modeling chemical processes using prior knowledge and neural networks. A.I.Ch.E.J. 40(8) (1994) 1328–1340
102. **Baughman, D.R.; Liu, Y.A.:** Neural networks in bioprocessing and chemical engineering. Academic press (1995) 320–325
103. **Ye, K.; Jin, S.; Shimizu, K.:** Fuzzy neural networks for the control of high-cell-density cell cultivation of recombinant Escherichia Coli. J. Ferm. Bioeng. 77 (1994) 663