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Research review paper

Control of fed-batch fermentations

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Abstract

Fed-batch fermentation is used to prevent or reduce substrate-associated growth inhibition by controlling nutrient supply. Here we review the advances in control of fed-batch fermentations. Simple exponential feeding and inferential methods are examined, as are newer methods based on fuzzy control and neural networks. Considerable interest has developed in these more advanced methods that hold promise for optimizing fed-batch techniques for complex fermentation systems.

Keywords: Fed-batch fermentation; Exponential feeding; Fuzzy control; Neural networks

Since the primary goal of fermentation research is the cost-effective production of bioproducts, it is important to develop a cultivation method that allows production of the desired product to a high concentration with high productivity and yield. Fed-batch culture has been widely employed for the production of various bioproducts including primary and secondary metabolites, proteins, and other biopolymers. During fed-batch cultivation, one or more nutrients are supplied to the fermentor while cells and products remain in the fermentor until the end of operation. Fed-batch is generally superior to batch processing and is especially beneficial when changing nutrient concentrations affect the productivity and yield of the desired product [1]. Since both overfeeding and underfeeding of nutrient is detrimental to cell growth and product formation, development of a suitable feeding strategy is critical in fed-batch cultivation. Various strategies have been developed to control the nutrient concentration within the optimal range, and have been applied to the high-cell-density culture of several microorganisms including *Escherichia coli* [2]. As Shimizu [3] pointed out, the control-system development for fermentation is not straightforward due to: (i) the lack of accurate models describing cell growth and product formation; (ii) the nonlinear nature of the bioprocess; (iii) the slow process response; and (iv) a deficiency of reliable on-line sensors for the quantification of key state variables.

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Nomenclature

$B(t)$	exponential cell growth term as a function of time t
CER	carbon dioxide evolution rate [mol/h]
DO	dissolved oxygen concentration [mol/L]
EtOH	ethanol concentration [mol/L]
F	nutrient (or substrate) feed rate [L/h]
$\overline{F}$	ideal nutrient feed rate [L/h]
$F_m(t)$	mass flow rate of carbon source at time t [g/h]
GD	glucose demand
GFR	glucose feed rate
GUR	glucose uptake rate [g/h]
GUR*	specific glucose uptake rate [g/g h]
k	proportionality parameter [g/mol]
m	specific maintenance coefficient [g/g h]
OD	optical density
OTR	oxygen transfer rate [mol/h]
OUR	oxygen uptake rate [mol/h]
P	product concentration [g/L]
Qo_2	specific respiration rate [mol O_2/g · h]
$q_{s,crit}$	specific sugar critical feed rate [g/g · h]
q_{sf}	specific nutrient (or substrate) feed rate [g substrate/g DCW · h]
$R_{o/g}$	ratio of OUR to GUR
RQ	respiratory quotient
s	nutrient(or substrate) concentration [g/L]
s_{crit}	critical nutrient concentration in the medium [g/L]
s_F	nutrient concentration in feeding solution [g/L]
s_{sp}	set point of nutrient concentration [g/L]
$s(t_1)$	nutrient concentration at time t_1 [g/L]
T	temperature
t	time [h]
t_0	initial time [h]
t_1	arbitrary time [h]
$V(t_0)$	culture volume at time t_0 [L]
$V(t_1)$	culture volume at time t_1 [L]
x	cell concentration [g/L]
$x(t_0)$	cell concentration at time t_0 [g/L]
$Y_{c/g}$	carbon dioxide yield
$Y_{x/gal}$	cell yield on galactose
$Y_{x/s}$	cell yield on nutrient(or substrate) [g/g DCW]

Greek Symbols

α	fuzzy factor
Δt	time step size [h]
ΔpH(t)	pH change at time t in Δt
$\Delta\mu(t)$	μ change at time t in Δt
μ	specific growth rate [h^{-1}]
μ_{sp}	set point of μ [h^{-1}]
λ	correction factor

Several variables are used for control purposes and can be classified as either measured or manipulated. Measured variables can be classified further as either directly measured or indirectly determined. Directly measured variables include temperature (T), pH, dissolved oxygen concentration (DO), optical density (OD), substrate concentration (s), pressure, and exit gas composition. These variables can be measured directly during cultivation by various instruments such as DO probes (DO), pH probes (pH), T probes (T), spectrophotometers (OD), high-performance liquid chromatography (HPLC) (*s*), glucose analyzers, gas chromatographs, and mass spectrometers. Indirectly determined variables include specific growth rate (μ), cell concentration (x), oxygen uptake rate (OUR), oxygen transfer rate (OTR), carbon dioxide evolution rate (CER), and respiratory quotient (RQ); indirect variables are estimated or calculated from one or more of the directly measured ones. The manipulated variables include agitation speed and substrate feed rate. Most of these variables have been used in combination to determine the nutrient feed rate during fed-batch cultures. Here we review the recent advances in development and application of control strategies for fed-batch cultivation.

1. Feed control strategies

1.1. Simple indirect feedback (single-loop) methods

Simple indirect feedback control schemes that couple nutrient feeding with measurement of pH (pH-stat) or DO (DO-stat) have been developed [2]. Fig. 1 is a block diagram for these schemes, where nutrient feed rate is manipulated to maintain pH or DO at the set point. When pH (or DO) becomes higher than its set point, the on/off controller feeds nutrient to the fermentor at a predetermined rate. The pH-stat with high limit is based on the fact that pH rises due to excretion of ammonium ions when the principal carbon substrate is depleted [4]. Similarly, the DO-stat is based on the fact that DO increases sharply when a key substrate is depleted [5].

1.2. Predetermined feeding strategies

Exponential feeding is a simple but powerful method that allows cells to grow at a constant specific growth rate. High-cell-density cultures of nonrecombinant and recombinant *E. coli* strains have been carried out using this method [2,6–8], and the method has been applied to other cultures. With *E. coli*, acetate production, which can inhibit cell growth as well as

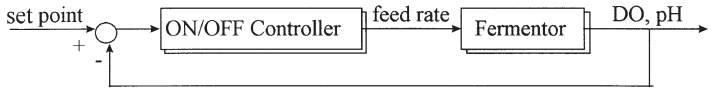

Fig. 1. Block diagram of indirect feedback (single-loop) method.

product formation, could be minimized by controlling the specific growth rate below the critical value (0.1–0.35 h^{-1} depending on the strain and growth medium) for acetate formation [2]. This feeding strategy does not use a measured variable for manipulating the nutrient feed rate. Instead, the controller manipulates the feed rate only by reference to the fermentation model equations. The nutrient feeding rate can be determined by Eq. (1), which is derived from a mass balance with the assumption of a constant cell yield on substrate and constant maintenance coefficient throughout the fermentation; thus

$$F_m(t) = \left(\frac{\mu}{Y_{X/S}} + m\right)x(t_0)V(t_0)e^{\mu(t-t_0)} \quad (1)$$

In Eq. (1), $F_m(t)$ is the mass flow rate of the carbon source at time t (g/h), μ is the specific growth rate (h^{-1}), $Y_{X/S}$ is the cell yield on carbon substrate (g Dry-Cell-Weight (DCW)/g), m is the specific maintenance coefficient (g/g DCW · h), t_0 is the time at which feeding is started, and $x(t_0)$ and $V(t_0)$ are cell concentration (g DCW/L) and culture volume (L) at t_0, respectively. Since this method assumes that cells grow exponentially with time, process disturbances and system nonlinearity cannot be tolerated when cell growth does not match the predetermined profile due to the overfeeding or underfeeding of nutrient. Of course, error detection can be incorporated into the control structure to raise an alarm if the expected and measured cell-mass profiles deviate significantly.

1.3. Nutrient feeding according to glucose uptake or demand

The concentration of carbon source in the culture medium can be controlled at a desired value if we can measure it on-line. As an example of this, Kim et al. [9] used as a glucose analyzer for fed-batch culture of *Alcaligenes eutrophus* for the production of poly(3-hydroxybutyrate). Glucose was controlled at a nominal concentration of 15 g/L. Even though actual glucose concentration fluctuated between 10 and 20 g/L, especially at low cell concentrations, the final cell and poly(3-hydroxybutyrate) concentrations obtained in 50 h were 164 and 121 g/L, respectively. This clearly shows that controlling nutrient concentration in an optimal range is an efficient way of cultivating cells to high concentration, even though this is a simple single-input/single-output (SISO) system. Due to the measurement time delay and instability of on-line glucose systems [10], methods that estimate and predict substrate consumption rate are generally preferred. Some of the common inferential control approaches are discussed below.

Konstantinov et al. [11] introduced the balanced DO-stat method. They measured the exit gas composition from the fermentor in real time, estimated the glucose uptake rate (GUR), and determined the nutrient (or glucose) feed rate F. This can be considered as a modified single-loop control method. They also employed an algorithm that guarantees sufficient oxygen supply during glucose feeding and that prevents glucose overfeeding.

Konstantinov et al. [12] employed the concept that glucose feed rate (GFR) should equal GUR in order to prevent glucose accumulation or depletion. Using this strategy, the cell and phenylalanine concentrations obtained in 56 h were 40 and 46 g/L, respectively, by fed-batch culture of *E. coli* AT2461. Several variables such as DO and exit gas compositions were measured to estimate specific state variables, namely OUR, CER, RQ, GUR, the ratio of OUR to GUR ($R_{o/g}$), and carbon dioxide yield ($Y_{c/g}$). Acetate excretion was minimized by adjusting the specific glucose uptake rate (GUR*), which decreased with time. The oxygen concentration was maintained constant by manipulating agitation speed and F.

Kleman et al. [13] developed a systematic methodology to determine F during the fed-batch culture of *E. coli* B. Their control scheme had two components: One predicted future glucose demand (GD) based on the recent value of F (feedforward component), and the other corrected the minor offsets (feedback component). The last five data points measured by an on-line glucose analyzer were regressed to determine GD at the next time interval. The error between the predicted and measured values was corrected by feedback adjustment of F. Using this strategy, a relatively high cell concentration of 65 g/L could be obtained in 9 h. It should be mentioned that oxygen supply became a problem at high cell density, and subsequently the measurement variables oscillated.

As an alternative to GD, Hosobuchi et al. [14] utilized the conversion rate of the nutrient[glucose + ML236B Na(compactin)] to determine feed rate. *Streptomyces carbophilus* was cultured to produce pravastatin in a 600-L fermentor [14]. Nutrient concentration was measured on-line by HPLC to estimate its conversion rate per unit volume, and the value at the next scan time was predicted by regression of the last five data points [14]. As before, the control rule had two components: the estimated conversion rate (feedforward component) and the deviation of ML236B-Na concentration from the set point (feedback component).

A feeding method based on on-line determined maximum substrate uptake rate has been employed by Oh et al. [15] for culture of recombinant *Saccharomyces cerevisiae* for producing extracellular glucoamylase. On-line measurements of dissolved oxygen and the substrate flow rate were the only parameters needed for implementing this control scheme, which is suited only to aerobic cultures.

Zhou et al. [16] determined the nutrient feed rate in the cultivation of hybridoma cell line MAK secreting immunoglobulin G (IgG) monoclonal antibody by the stoichiometric relation between glucose and oxygen consumption [see Eq. (2)]; thus,

$$F = \frac{\text{O}_2 \text{ consumption at present time step}}{\text{O}_2 \text{ consumption at previous step}} \cdot \frac{\text{glucose consumption at previous time step}}{s_F \Delta t} \tag{2}$$

The OUR was determined once every hour to control the glucose concentration at its set point. In the fed-batch culture, the accumulation of lactate that inhibits cell growth was reduced, and the growth phase was extended. In comparison with the batch culture results (2×10^6 cells/mL), a much higher cell concentration (1.36×10^7 cells/mL) with a 90% viability could be obtained.

A simplified block diagram for the above four cases is shown in Fig. 2. Several variables such as DO, exit gas composition, and nutrient concentration are measured, allowing estimation of GUR, GD, and nutrient conversion rate from the model equations. A classical on/off

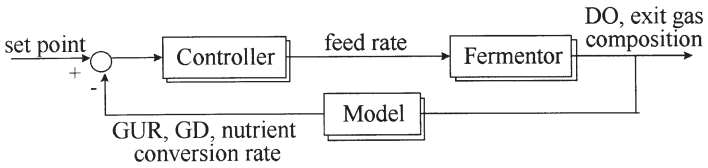

Fig. 2. Block diagram of nutrient feeding strategy using GUR or GD estimation.

or Proportional-Integral-Derivative (PID) controller is then used to manipulate nutrient feed rate to maintain these variables at their set points. Adaptation can be incorporated into the controller by allowing model parameters to be time variant.

1.4. Nutrient feeding according to inferred substrate concentration or specific growth rate

Even though a few on-line sensors have been developed and employed in fermentation, their widespread use has been hampered by several problems including poor thermal stability during sterilization (e.g., enzyme electrodes), poor reliability, or a high level of complexity [e.g., filtration type systems and flow injection analysis (FIA) systems]. Liu et al. [17] proposed a method that estimates glucose concentration by integrating the amount of nutrient added to the fermentor and the CER. *Brevibacterium flavum* was cultivated to produce 92 g/L of L-lysine in 72 h by manipulation of the feed rate. As with other inferential control methods, this control strategy consists of two components: system estimation and control. Wipf et al. [18] measured other variables such as inlet airflow rate, culture volume, and CO_2 content to estimate glucose concentration for the production of α-interferon by recombinant *E. coli*. Somewhat surprisingly, the large-scale (1,000-L) fermentation system was successfully controlled by this method. In another study, Massimo et al. [19] estimate specific growth rate from OUR data based on a neural network model for the production of penicillin. Specific growth rate was controlled at a low value in order to optimize penicillin production. Specific growth rate could also be estimated by training the neural network (see later), but there were some problems. Specifically, it was difficult to determine an "optimal" network topology and to establish the methodology for determining the stability of the neural network model. The concept for each of these three cases is illustrated in Fig. 3. From the measured CER or OUR, variables such as nutrient concentration and specific growth rate are estimated by an appropriate model. A classical controller can then be used to control feed rate on-line and to maintain measured variables at their set point. Once again, an adaptive control mechanism is made possible by allowing the model parameters to be time varying, or by incorporating an autotuning adaptive control loop.

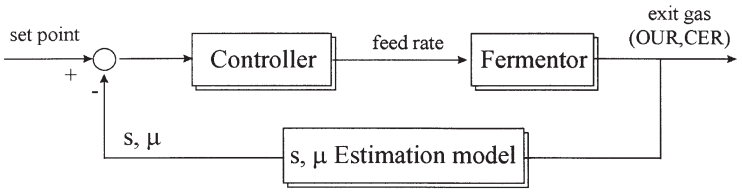

Fig. 3. Block diagram of feeding strategy utilizing estimated variables.

1.5. Other methods

The preceding inferential methods estimate a specific state or state-related variable from easily measured variables. Feedback control is then effected to maintain the calculated variable at some predetermined value (e.g., to meet an estimated glucose demand or to maintain a fixed substrate concentration). Adaptation is possible by allowing parameters in the estimation model or control parameters to be time variant.

A variety of alternative feed control strategies have also been employed, some based on simple models of the process. In such cases, an accurate model of the biological system is a prerequisite for accurate control, although some modeling error can be tolerated if on-line adaptation is employed.

Yamane et al. [20] developed a method to determine feed rate F from on-line measures of culture volume V calculated from the total amount of the nutrient solution added and the cell concentration x (from on-line turbidity) during cultivation of recombinant *E. coli* HB101. The feed rate was directly calculated from Eqs. (3) and (4) for a preset specific growth rate and yield coefficient; thus,

$$F = \frac{q_{sf} V x}{s_F} \tag{3}$$

where

$$q_{sf} = \frac{\mu}{Y_{X/S}} \tag{4}$$

The specific substrate feed rate, q_{sf}, was considered to be time invariant.

An adaptive strategy was suggested by using time-varying values of q_{sf}. A similar strategy was employed for the production of α-amylase using recombinant *E. coli* JM107(pQR126) [21]. In this study, galactose and acetate concentrations were monitored on-line by HPLC. The prefixed feed rate trajectory based on Eq. (3) was modified to address problems of reduced productivity at high growth rates; the modified equation is presented as Eq. (5).

$$F = \frac{\mu}{Y_{x/gal}} B(t) R \tag{5}$$

where

$$B(t) = B(t - \Delta t) e^{\mu \Delta t}$$

and

$$R = \begin{cases} 0.5 \text{ if a critical analyte accumulates} \\ 1 \text{ if the critical analyte concentation is low} \end{cases}$$

$B(t)$ is the biomass estimate for the time t. The scaling factor R was set at 0.5 if the acetate concentration (s_{acet}) exceeded the threshold of 0.5 g/L, and 1 if not. This method resulted in large fluctuation in s_{acet} and s_{gal} (galactose concentration) and, therefore, was modified once again. First, galactose was chosen instead of acetate as an analyte critical to cell growth. Then, μ was increased from 0.2 to 0.45 h^{-1} with a rate of 0.025 h^{-2} if s_{gal} was below 0.25 g/L.

In addition, if s_{gal} was higher then 0.4 g/L, $B(t)$ was reduced by 5% over 12 min. Final cell mass increased a little, but the latter method reduced the fluctuation of s_{gal}, s_{acet}, and F.

In another study, a feedforward-feedback control system was developed to regulate the feed rate of nutrient for the production of glutathione (GSH) by *S. cerevisiae* KY6186 [22]. Exit gas from the fermentor was analyzed to calculate the reference profile of F (feedforward), and the current ethanol concentration was measured to modify this profile (feedback).

Exit gas analysis was also employed by another group in a somewhat different way. Kim et al. [9] inferred CER from the exit gas composition and calculated the feed rate F using Eq. (6).

$$F = \frac{[s_{sp} - s(t_1)]V(t_1) + \mathrm{GUR}t_1\Delta t}{(s_F - s_{sp})\Delta t} \tag{6}$$

Using this feeding strategy, cell and poly(3-hydroxybutyrate) concentrations of 124 and 92 g/L, respectively, were obtained in 49 h. The GUR in Eq. (6) equals the CER multiplied by a proportionality parameter k.

Recently, Kurtz et al. [23] developed a nonlinear controller from a simplified dynamic model and a nonlinear observer predicting cell and substrate concentrations on-line in a competitive mixed-culture fermentation. Even though the fermentation was carried out by recycling and purifying culture medium—a methodology different from fed-batch type culture—the unmeasurable variables could be predicted mathematically. An extended Kalman filter for estimation and prediction of culture states has been applied also to batch beverage fermentation [24].

1.6. Manipulation of feed rate using fuzzy control and neural networks

1.6.1. Basic concepts of fuzzy inference

When the conventional deterministic modeling and control methodologies perform the yes-or-no-type calculation, all the situations of real systems need to be described precisely by crisp numbers [25]. However, this information is often uncertain and may not be available. Fuzzy set theory was first proposed in 1965 by Zadeh to deal with such uncertainty or "fuzziness" of real situations [25,26]. Fuzzy control is based on this fuzzy set theory.

There are various complex industrial processes that are too fuzzy to be controlled by conventional deterministic control schemes: fermentation, batch chemical reaction, activated-sludge process, rotary cement kiln process, and so on [26]. Fuzziness is mainly due to the nonlinearity in system behavior and is also due to poor measurements. Benefits of applying fuzzy control to such processes are: (i) implementation of control based on expert knowledge and (ii) a robust control performance [27]. The fuzzy control scheme is depicted in Fig. 4.

Determination of control actions from the fuzzy rules employs three main steps: fuzzification, reasoning, and defuzzification [26,27]. Most of all, crisp values of measured data are first normalized and converted to linguistic values such as high/low and big/small. In a single-input/single-output process, two kinds of variables are defuzzified: error (E) and change of error (CE) [26]. Then, for instance, each variable has a fuzzy value such as PB (positive big), PS (positive small), ZE (zero), NS (negative small), and so on. Next, by the prescribed rules, membership values of control input variable (U) are determined as linguistic values. Fuzzy rules have the form of conditional statements [26] such as

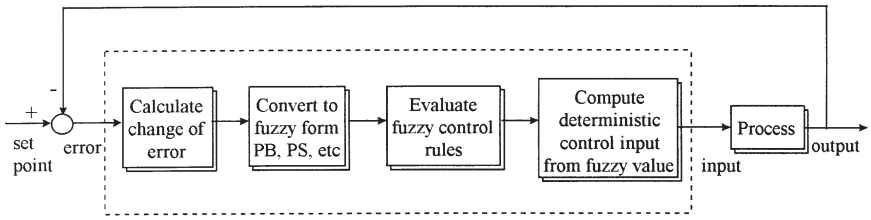

Fig. 4. Control system using fuzzy rules [26].

- Rule 1: If (E = PB and CE = PS) then U = NS.
- Rule 2: If (E = NB and CE = NS) then U = PS.

A series of rules can be listed as a rule table with corresponding error and change of error. For given E and CE, the inference engine determines membership function values of all the rules.

Finally, such linguistic membership values are defuzzified to give a control input value, which is denormalized to be crisp. There have been several well-known defuzzification methods: (i) center-of-gravity defuzzification; (ii) center-of-sums defuzzification; (iii) center-of-largest-area defuzzification; (iv) first-of-maxima defuzzification; (v) middle-of-maxima defuzzification; and (vi) height defuzzification [27]. The best choice may be different for different systems, and there are some criteria for selecting a defuzzification method by taking into account the characteristics of the method. Some characteristics that need considering are: (i) continuity; (ii) disambiguity; (iii) plausibility; and (iv) computational complexity [26,27]. Further information on fuzzy control theory can be found in recent books and reviews [25–28].

1.6.2. Applications of fuzzy inference to bioprocess control and modeling

During the last several years, fuzzy inference has been applied to various fermentation processes. To understand the reasons for this extensive development of fuzzy sets, two main aspects need to be mentioned [26]. First, the notion of a fuzzy set a tool for modeling intermediate grades of belonging that occur in any concept is very attractive, especially from an application point of view as mentioned previously. Second, a variety of tools incorporated in the framework of fuzzy sets enable us to find a suitable concept to cope with reality.

Kitsuta and Kisimoto [29] studied the control of glutamic acid production by *Brevibacterium* species. In their study, a deterministic model for deciding the quasi-optimal policy could not be constructed easily because of a lack of quantitative information on the effect of sugar concentration and hence the mechanism of glutamic acid production. Therefore, deterministic computer control was not practical for the whole fermentation time course, even if parameters were completely identified. The molasses feeding policy and the time of penicillin addition significantly affected glutamic acid production in this culture. In particular, the production of glutamic acid was reduced significantly by sugar starvation at the beginning of the production phase. A fuzzy supervisory control scheme was developed to address this problem. The transition from the early period of the production phase to the later period was identified using the inference engine of the control system. In the early production phase, ex-

cess feeding of molasses followed the best manual feeding strategy that had been identified through earlier experimentation. In the later production phase, the molasses feed was regulated by the PID controller. To demonstrate better performance, this methodology was compared with three other schemes—the computer-free control, PID control of CO_2 concentration in the exit gas by regulating the molasses feed rate, and PID control with some heuristics. In a 5-L jar fermentor experiments, the fuzzy control method led to the highest concentration (75 g/L) of glutamic acid.

Another rule-based fuzzy logic control system was developed and simulated for the control of penicillin concentration in a fed-batch bioreactor by Nyttle and Chidambaram [30]. The control error in the product concentration and the nutrient feed flow rate *F* were used as the linguistic variables, and *F* was manipulated to control product concentration (*P*). After several simulation runs, a set of six rules was constructed. To test the robustness of this fuzzy logic control system, several parameters were varied: the initial feed flow rate, initial reactor volume, and the sampling time. The effect of measurement noise on *P* was also studied. Following all of this work, the authors recommended a simple fuzzy controller for the available case of on-line measurement of *P*.

There have been many strategies to prevent the crabtree effect, but the control of ethanol concentration to maintain a zero net production is one of the more commonly used solutions because of the low cost of ethanol sensors. To do this in fed-batch culture by a conventional Proportional-Integral (PI) controller, integral action is very important, and adaptive or scheduled alteration of the control parameters may be needed to deal with the dynamic nonlinear behavior of the process. In many cases, the intervention of a human operator with experienced knowledge is helpful, and use of fuzzy logic controller (FLC) is indicated even though other effective methods for maintaining a low level of ethanol have been demonstrated [31,32].

A classical FLC uses control error and its rate of change in the premise part of the linguistic rules. This approach works well when the initial error in measured ethanol concentration is very small. However, when the initial error is large, controller overreaction can result in an overshoot. Alfafara et al. [33] used a modified conventional FLC (Fig. 5) to get around this problem. A two-step solution was used. First, the diagnostic determination of “glucose emergency states (its accumulation or deficiency)” was adapted to avoid controller overreaction. Glucose feed rate was manipulated by a modified feedforward-feedback controller. The actual glucose feed rate was the sum of a nominal feed rate given by the programmed controller based on model equations, and a compensation feed rate is given by the precompensator. This precompensator could be a PI or fuzzy controller, and the identification of emergency

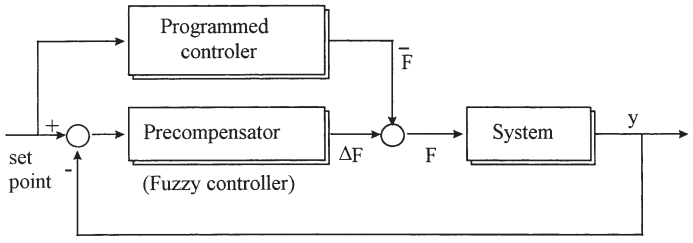

Fig. 5. Structure of the feedforward-feedback controller [33].

states was adapted to it. Then, with information on optimal specific growth rate, glutathione production was maximized while ethanol concentration was maintained constant. This control scheme gave better performance than the classical approach.

Zhang et al. [34] suggested the concept of a functional state in the aerobic fed-batch culture of baker's yeast, and demonstrated the application of fuzzy logic to control this process. The process was first divided into four functional states according to the different metabolic phases: (i) the first ethanol production state; (ii) the mixed oxidative state; (iii) the complete sugar oxidative state; and (iv) the second ethanol production state. The objective of control was to maintain state (iii), which was satisfied when the respiratory quotient (RQ) was maintained at unity. Unlike other fuzzy controllers that specify the feed rate F directly, a fuzzy rule was used to modify the conventional exponential feeding strategy. From the model equations, the "ideal" substrate feed rate $\bar{F}$ was calculated as shown in Eq. (7):

$$\bar{F} = \frac{q_{s,crit} x V}{(s_{in} - s_{crit})} \tag{7}$$

A positive fuzzy factor α was then used to connect this "ideal" feed rate to the actual feed rate F as shown in Eq. (8):

$$F = \begin{cases} (1+\alpha)\bar{F} \text{ if RQ} \le 1 \\ (1-\alpha)\bar{F} \text{ if RQ} > 1 \end{cases} \tag{8}$$

The value of the fuzzy factor α depended on the RQ and its rate of change.

Another fuzzy control application to a fed-batch culture was for the production of coenzyme Q_{10} [35]. This process was carried out under oxygen supply limitation (i.e., it was controlled by the oxygen supply when substrates were fully supplied). When the dissolved oxygen concentration in the fermentation broth reduced to almost zero, reduction-oxidation (or redox) potential values reached a constant level. In this situation, the productivity of coenzyme Q_{10} and the cell concentration (measured using turbidimeter) were regulated mainly by the rate of oxygen supply. Therefore, the oxygen supply was selected as a manipulated variable in the fuzzy controller rather than the feed rate. Key inputs to the fuzzy controller were optical density, specific growth rate, and fermentation time. Stable productivity and cell concentration were maintained by this control system in a 30-L jar fermentor.

Fuzzy modeling methods based on fuzzy set input rather than numerical input have been applied to the modeling of fermentation processes since the early 1980s and have attracted special interest recently. One modeling method using fuzzy logic was developed by Jitsufuchi et al. [36]. In their study, a fuzzy model was formulated for describing the self-growth inhibition of *Zymomonas mobilis* Although deterministic models exist, their application is limited by nonlinearity and a complicated mechanism of reaction. To improve such models, coefficients of models were determined by fuzzy reasoning. *Z. mobilis* produces ethanol in an oxygen-free environment, but the production of acetaldehyde rises as DO increases. *Z. mobilis* growth is inhibited by the ethanol concentrations greater than 77 g/L or by the acetaldehyde concentrations (A_{in}) greater than 2 g/L. Fuzzy rules were developed using this information. Inputs such as A_{in} and DO were used to determine the model coefficients and even the growth rate. This fuzzy model gave closer estimates than those obtained from a linear

(deterministic) model of the experimental data. Kishimoto et al. [37] studied the culture phases of the same system. An algorithm for clustering culture status into several phases (e.g., lag growth, transient, and production) was developed.

Following these studies, research by Terhi et al. [38] focused on fault diagnosis and the control of yeast cultures with a real-time fuzzy-knowledge-based system. In an industrial fed-batch baker's yeast production system, the substrate feed rate profile was controlled conventionally on the basis of empirical knowledge. By employing a fuzzy phase recognition method, sudden changes in culture state that are often detrimental in a fermentation process could be avoided, and the process models used for control were considerably simplified even when they represented only a part of the process at a time.

Fuzzy logic is also routinely employed to handle uncertainties in both the knowledge and the measurements. On the basis of measured data, the expert system recognizes the current process phase, possible faults, and any need for control action. The process knowledge is stored in a two-level hierarchical knowledge base and in fuzzy process variables. For on-line process control, the system first recognizes the current process phase on the basis of top-level rules in the knowledge base. Then, according to the results of process diagnosis based on measured data, an appropriate control strategy is inferred making use of lower level rules describing the process during the unknown phase. During each process phase, the most critical process variables are checked in order to identify possible failure situations and the need for easy control actions. With fuzzy logic, it is possible to model and recognize process phases, to change control strategies, and to change fuzzy sets on-line according to the process dynamics.

Another group developed a fuzzy recognition model for the definition of culture state. The main function of the model by Fu et al. [39] was to predict and determine the feed timing for acetyl spiramycin fermentation process. Although many variables were controlled typically, the substrate concentration was the most important over most of the time course of the fermentation because the product was a secondary metabolite (antibiotic) synthesized by the action of cell enzymes under various specific nutrient regimes. The feed rate of substrates during the culture process needed to be controlled according to changes in metabolism. Excessive or early feeding can disturb the cell metabolic balance, while insufficient or late feeding can reduce growth. The timing and amount of feed are usually determined by experience to maintain substrate composition within a suitable range. Instead of this scheme, Fu et al. [39] constructed a fuzzy model and control system to obtain an optimal feeding strategy in an industrial process for producing acetyl spiramycin. The system guaranteed a stable production and yield of the antibiotic. The control procedure was as follows: (i) sample the culture and analyze the off-line measurements; (ii) judge which pattern class the running process is in; (iii) calculate the fuzzy membership values; and (iv) specify the feed rate. This method gave better performance and product yield in a series of fermentation runs. Fuzzy prediction of fermentation time for a commercial brewery has also been attempted [40].

1.6.3. Neural networks: Basic concepts and applications for control of bioprocesses

Problems dealing with real processes or real situations have generated several new ideas other than fuzzy theory in modeling and control area: The neural network concept is one such development. Neural networks imitate the human brain function [41,42] that depends on in-

formation storage in the neuron connection pattern. There are several advantages of using neural networks in modeling and control of real processes [41]. Neural networks can deal with nonlinear and multivariate systems, and can learn or adapt process data in their structure. Because of these characteristics, neural networks have been applied to many different areas. Information on the application of neural networks to various chemical processes can be found elsewhere [43,44]. The application potential of neural networks comes from their special structure and data adaptation ability. In fact, an artificial neural network preserves process information on the connections between artificial neurons or "nodes" by a selected training or leaning algorithm [44].

A neural network is depicted in Fig. 6(a). The circles in Fig. 6(a) represent nodes that are arranged in three layers; the nodes are interconnected as shown. Fig. 6(b) shows the calculation procedures at each node with the input values coming from the previous layer of nodes. If we define node inputs as x_i (i-th input) and weighting factors as w_i (i-th factor), this calculation can be formulated as shown in Eqs. (9) and (10) [34,45,46]:

$$\text{Sum} = \sum x_i \cdot w_i \tag{9}$$

$$\text{Neuron output} = f(\text{Sum}),\ [f(\cdot)\text{: activation function}] \tag{10}$$

where the activation function can be selected by the specific applications.

To store and adapt process data, nodes and connections are combined to form a structure or "topology." The performance of neural networks in modeling and control systems depends on the number of layers and nodes in each layer. Therefore, many kinds of neural networks have been studied. For fermentation processes, the feedforward structure has been employed especially frequently [42,45–49]. Multilayer and recurrent neural networks are the most popular types and are based on a sigmoidal activation function [46,48,50,51]. Other topologies are based differently. For example, the radial basis function network [47] is based on a linear combination of Gaussian activation functions. The direct inverse model neuro-control system has a basic and general form, which learns the inverse model of the system [52]. Other neuro-control algorithms, such as the model reference adaptive, the predictive, and the feedback error designs are also utilized for many systems. These various network algorithms have been used in bioprocesses and biosystems.

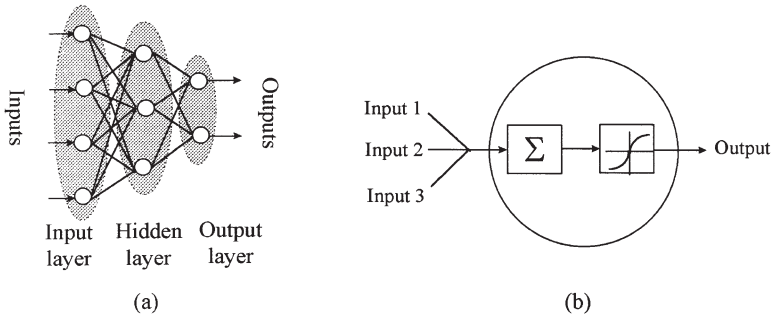

Fig. 6. Structure of neural networks: (a) feedforward network structure and (b) data processing at a neuron.

After selecting the topology, the network is "trained" on process data. There are two types of network training or learning algorithms: supervised and unsupervised. The supervised learning method is used when a set of input-output data is presented to the network and the weighting values are determined to closely match the network output to the measured output. Therefore, this type provides an advantage of having external references. However, in the unsupervised mode, weights are determined so that the network gives similar outputs for inputs, and this has no reference [41,43]. Sets of input and output data are learned or trained by neural networks to give proper outputs from the given inputs. This is done mainly by changing the weighting factors of all the connections by some learning algorithm [41]. Additional information on modeling or control systems using neural networks is available [41, 43,53].

A remarkable study of neural network-based control in fermentation systems was conducted by Boškovic and Narendra [47], who compared the performances of linear, nonlinear, and neural net controllers for fed-batch cultures. Through computer simulation of a baker's yeast fed-batch fermentation under identical conditions for each control scheme, they showed that the neural network system was better for the case where little data on process dynamics were available. When a fixed nonlinear controller or a nonlinear adaptive controller was applied, the best possible response was achieved, but those control schemes were not realistic because of the need for extensive prior information. Much less prior information was needed for the design of linear adaptive controllers, but the response was only marginally acceptable. However, the neural network adaptive controller gave acceptable response while using the same amount of prior information as the linear adaptive controllers. In addition, two different network basis functions were compared for use with the adaptive control scheme. Each estimator could predict the concentration of current biomass, glucose, and ethanol every 15 min using on-line measurements of temperature, redox potential, $\%CO_2$ released, and OD as inputs. Each network model learned several data sets that were measured at constant temperature; each network model was tested on temperature-varying data sets. The performance of the recurrent topology, with a sum of square error (SSE) of 0.06, was better than that of feedforward topology with an SSE of 0.14.

Recently, Tholudur and Ramirez [51] conducted a study of neural network-based schemes for optimizing fed-batch bioreactors. A neural network model was combined with basic material and energy balances to give an improved estimate of system dynamics. Two fed-batch bioreactor schemes were considered (yeast and bacterial). This technique provided a fast and reliable way of optimizing system performance, although incomplete state information resulted in suboptimal feeding policies.

In many applications, neural networks are used also to estimate state variables that are difficult to measure. Dynamic neural network models have been studied for the batch culture of yeast to estimate medium concentration. Teissier et al. [48] tried to achieve real-time estimation and prediction of yeast concentration during growth using a model based on a recurrent neural network topology, which uses the previous outputs as inputs at the current time step. In their study, yeast concentration was estimated from the initial cell concentration and the released CO_2 measurements with errors lower than 3% ($\pm 1.7 \times 10^6$ yeast cells/mL). Final cell concentration was predicted from only the initial condition to an accuracy of about $\pm 3 \times 10^6$ yeast cells/mL.

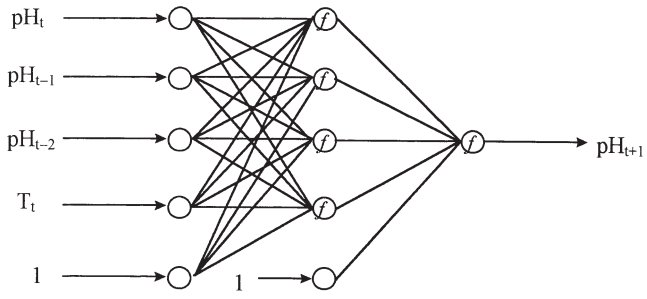

Fig. 7. Form of a one-step-ahead neural network predictor: f represents an activation function [45].

Latrille et al. [45] developed a neural network model (Fig. 7) to predict pH on-line for a process for producing fermented milk. A dynamic model was obtained from a two-step procedure using a recurrent neural network that predicted the total fermentation time accurately. First, a reference fermentation curve (pH vs. time) was obtained. Then, a recurrent neural network model was derived with three previous pH data points, current temperature, and current time as inputs. This method was applied to the fermentation of *Streptococcus thermophilus* ST23 in skim milk at several temperatures. The resulting neural net system predicted final fermentation time with a mean relative error of 7.7%.

The recurrent neural network design has been employed in another system. Karim and Rivera [50] tested this and feedforward-type networks on *Z. mobilis* fermentation for process state estimation. Sample configurations for state estimation employing feedforward and recurrent neural networks are shown in Fig. 8.

Glassey et al. [54] used neural networks as on-line estimators for biomass, recombinant protein concentration, and plasmid structural instability during the production of some proteins expressed by constitutive, chemo- and thermo-inducible vectors in recombinant *E. coli*. Cell concentration, protein concentration, and specific growth rate were estimated from

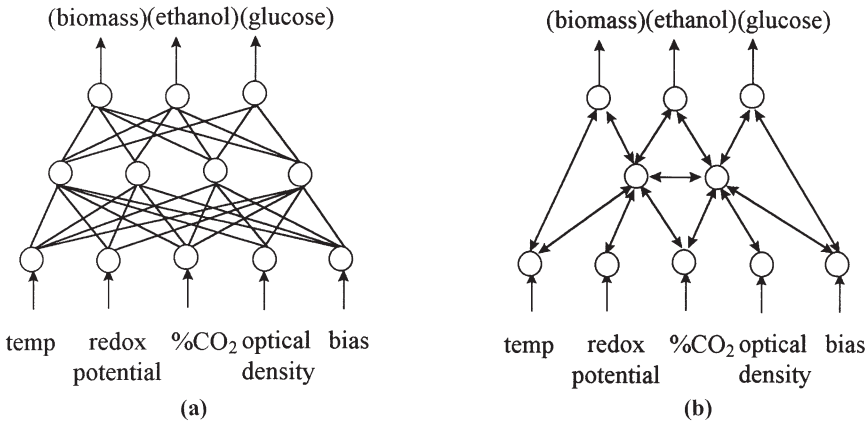

Fig. 8. Sample configurations for neural-based state estimation of ethanol fermentation [50]: (a) feedforward neural network and (b) recurrent neural network.

CER, OUR, and culture age. Nutrient feed rate was then calculated with a feedforward design. In that study, cell and protein concentrations were successfully estimated by the complicated network topology structures examined.

An application for the on-line prediction of some culture medium concentrations was studied by Breusegem et al. [49]. They proposed an adaptive algorithm in the neural model in which a sliding-window learning scheme was used to refresh the integrated knowledge. The sliding-window learning scheme limits the range of data used for learning. Moreover, instead of the commonly used backpropagation algorithm, a quasi-Newton method that converges more rapidly to an accurate solution was used to adapt the weights of the neural network. This resulted in an adaptive neural model that was updated at each sampling instant. With this model, the concentrations of substrate and cells at the next time step were predicted from several inputs of previous data and the dilution rate for the simulated Continuous-Stirred-Tank-Reactor (CSTR) system. This neural model adaptive scheme reacted rapidly to abrupt changes in the kinetics of the fermentation system and estimated concentrations accurately. Performance was compared with that of the extended Kalman filter [42]. The neural-adaptive system gave better results.

As we mentioned earlier, fuzzy control is regarded as a good choice for the control of fed-batch culture since it requires less knowledge of the actual process [55]. However, it takes too much time to decide the IF-THEN type of linguistic rules and membership functions [56]. To overcome the difficulties associated with conventional fuzzy control, several researchers have utilized neural networks to modify the fuzzy rules.

A feedforward-feedback control algorithm was applied by Ye et al. [55] to produce β-galactosidase with recombinant *E. coli*. The exponential feeding policy, Eq. (1), could be rewritten as Eq. (11):

$$\bar{F} = \frac{\mu_{sp} x V}{Y_{x/s} s_F} \tag{11}$$

This feedback element was corrected by the feedback component using a factor λ [see Eq. (12); thus

$$F = \bar{F}(1 + \lambda) \tag{12}$$

The λ value was determined from $\Delta\mu(t)$ and $\Delta\mathrm{pH}(t)$ using a fuzzy neural network (FNN). Ye et al. [55] observed relations between a set of state variables and a set of control variables, and this kind of relationship can be represented by the multilayer network structure. Such structure was trained with the experimental data. With this algorithm, a final cell density of 84 g/L was obtained, which was considerably higher than that obtained without using a fuzzy neural network (29 g/L).

Shi and Shimizu [56] studied a different method of combining neural network and fuzzy concepts. In *S. cerevisiae* fermentation, they improved the performance of the fuzzy controller with a neural network. DO and ethanol concentration (EtOH) were measured to calculate *F* by fuzzy rules. The changing patterns of DO and EtOH were recognized by a neural network, and *F* was modified in response to these changes. The block diagram for this system is shown in Fig. 9.

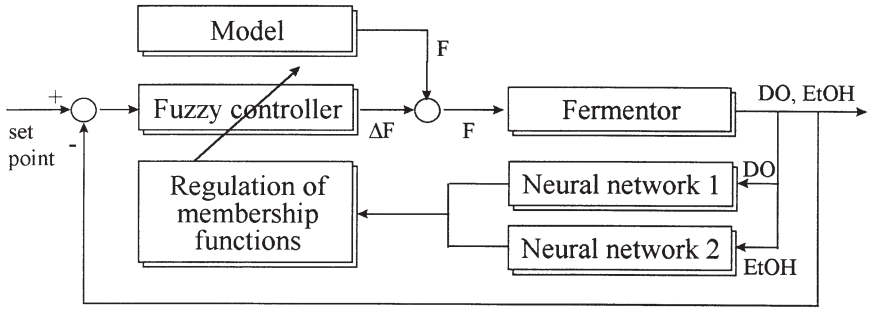

Fig. 9. Block diagram of a neuro-fuzzy system [56].

A neural network was applied also to the supervisory control of a lab-scale (2 L) *Bacillus thuringiensis* fermentation system [57] with the control objective of maximizing growth rate during the exponential growth phase. The neural network estimator was trained with a set of historic process data obtained from 18 fermentation experiments. Input data sets consisted of the type of inoculum, the accumulated process time, optical density of the growth medium, medium temperature and pH, and the dissolved oxygen concentration measured at each hour. The first trial employed a network giving only one output: OD at the next time step, Fig. 10(a). However, this resulted in imprecise estimation because the network had real-value inputs but normalized outputs, and it gave a poor prediction of the decline in OD in stationary phase. To overcome this problem, a network was constructed to give several "grade" values. These were then used as the inputs to fuzzy rules to give optical density at the next time step, Fig. 10(b). The predicted ODs matched the measured values well, and the mean square error was reduced to 0.293 (about 2%). PID controller set points for medium temperature and pH were then selected according to the predicted ODs. In this sense, the neural network system became a part of a larger adaptive control system.

Shimizu et al. [58] studied on-line state recognition in a yeast fed-batch culture with neural networks. In their case, there was no information about the classification of physiological states (cell growth and ethanol production) according to the substrate feeding rate. By ana-

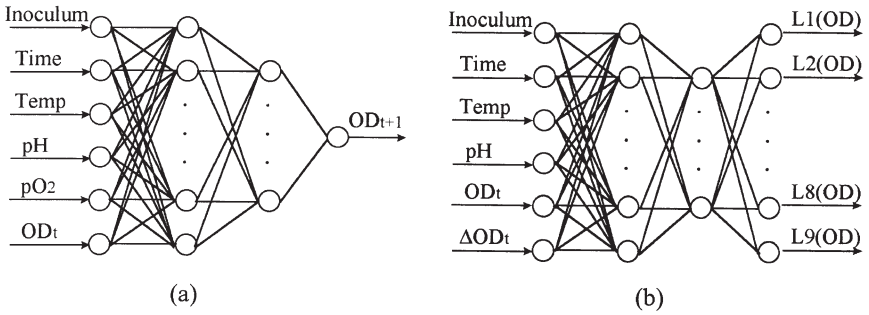

Fig. 10. Topology of a neural network [57]: (a) with a normalized real output pattern and (b) with a qualitative grade output pattern.

lyzing the pattern of error vectors from the mass balance equations, physiological states were classified, and fuzzy membership functions were constructed. Physiological states representing the activities of cell growth and ethanol production are thought to be related to the mole fluxes and energy parameter values in the metabolic pathways, and can be calculated from a model based on the pathways. With this model, the time-dependent metabolic parameter P/O (oxidative phosphorylation) was adaptively estimated to give current cell growth rates. From the error vector, it was possible to recognize whether there are more kinds of reactants and/or products, or whether there is a delay in the enzyme reactions. The recognition results showed that probability of correct state estimation was greater than 80%.

2. Concluding remarks

As discussed in this review, several approaches are available for the control of fed-batch cultures. The control of such systems aims mainly to maintain an appropriate substrate feed rate to prevent overfeeding or underfeeding. Available control methods include the simple exponential feeding strategies and inferential control schemes with classical control action. The newer knowledge-based control systems—e.g., those relying on fuzzy logic and neural network concepts—are receiving considerable research attention with promising results.

Acknowledgments

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