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# A modular simulation package for fed-batch fermentation: penicillin production

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**Abstract**

Simulation software based on a detailed unstructured model for penicillin production in a fed-batch fermentor has been developed. The model extends the mechanistic model of Bajpai and Reuss by adding input variables such as pH, temperature, aeration rate, agitation power, and feed flow rate of substrate and introducing the $CO_2$ evolution term. The simulation package was then used for monitoring and fault diagnosis of a typical penicillin fermentation process. The simulator developed may be used for both research and educational purposes and is available at the web site: http://www.chee.iit.edu/ ~ control/software.html.


*Keywords:* Penicillin fermentation; Modeling and simulation; Educational software

## 1. Introduction

Industrial challenge problems provided by various industrial researchers at the 1990 AIChE Annual Meeting, Session 24 have been very useful in testing new process monitoring, fault diagnosis, and process control methods proposed by academic researchers. Notably, the Tennessee Eastman Plant-wide Industrial Control Problem (Downs & Vogel, 1993) has become an important testbed referenced in more that 100 research publications on process monitoring and control studies. The purpose of this contribution is to share a testbed for fed-batch fermentation processes developed by using a realistic dynamic model of penicillin fermentation. The aim of this work is to build a mathematical model detailed enough to be used as a testbed for several applications such as process monitoring and empirical model development and develop the software so that it can be readily used by others. No claim is made that the model can be used for modeling strain improvements or explaining fundamental phenomena in penicillin fermentation. A detailed fundamental model on penicillin production and its applications based on a morphologically structured model which may be more suitable for such studies, can be found elsewhere (Birol, Undey, Parulekar & Cinar, 2002). The authors have used the model and software developed for testing batch process monitoring based on multivariate statistical methods and fault diagnosis (Cinar & Undey, 1999; Undey, Tatara, Williams, Birol & Cinar, 2000), and are currently working on testing model predictive control strategies. As the importance of batch processes in chemical and biotech process industries has increased in recent years, such dynamic models would be useful in testing modeling, monitoring, diagnosis, and control techniques proposed by researchers and in case studies in process control courses. A Web site is established (http://www.chee.iit.edu/ ~ control/software.html) to distribute the software, and posted instructions for using the software, updates, and extensions made by the authors and other researchers. Comments from academic and industrial researchers resulted in significant improvements of the model and software. However, all simplifications and remaining errors are the responsibility of the authors.

Production of secondary metabolites has been the subject of many studies because of its academic and industrial importance (Atkinson & Mavituna, 1991).

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Filamentous microorganisms are used commercially for the production of secondary metabolites such as antibiotics. The formation of the target product, the antibiotic, is usually not associated with cell growth. For this reason, it is common practice to grow the cells in a batch culture followed by a fed-batch operation to promote the synthesis of the antibiotic. In this study, penicillin production is considered due to its nonlinear dynamics and multistage nature as well as its industrial importance. There is an extensive literature on modeling of penicillin production with varying degrees of complexity (Constantinides, Spencer & Gaden, 1970; Heijnen, Roels & Stouthamer, 1979; Bajpai & Reuss, 1980; Nestaas & Wang, 1983; Menezes, Alves, Lemos & Azevedo, 1994). The models reported may be grouped as structured and unstructured models. In unstructured models, all cellular physiology information are gathered into a single biomass term so that there is no explicit structural information left about the cellular activity resulting in a rather simpler model. The structured models for penicillin production on the other hand include the effects of cell physiology on penicillin production by taking into account the physiology and differentiation of the cell change along the length of the hyphae and during fermentation.

The mechanistic model of Bajpai and Reuss (1980), the segregated model of Nestaas and Wang (1983) and the model of Heijnen et al. (1979) are some of the frequently cited unstructured models. A detailed comparative discussion of unstructured mathematical models can be found in Menezes et al. (1994). Some of these models do not consider the effects of operating variables such as pH, temperature, aeration rate, agitation power, feed flow rate of substrate on biomass growth and penicillin production. Others do not have all the biomass growth, $CO_2$ and penicillin production, substrate (both carbon source and oxygen) consumption, and heat generation terms in model equations. In this study, we have used experimental data available in literature (Pirt & Righoletto, 1967; Metz, Bruijin & van Suijdam, 1981) to improve the simulation of penicillin production by extending the existing mathematical models. The mechanistic model of Bajpai and Reuss (1980) was utilized as the basis of our modeling efforts. The effects of environmental variables such as pH and temperature, and input variables such as aeration rate, agitation power, feed flow rate of substrate on biomass formation have been included in the model for completeness. This software provides flexibility to test the feeding strategies for addition of sugar into the bioreactor to produce penicillin. Feeding strategy related work has been well documented for penicillin production (Bajpai & Reuss, 1981). The addition of sugar can be carried out either batchwise several times during the run (as series of batches) or continuously or as a fed-batch process. There are a number of policies that can be employed with the aim of optimizing penicillin production, (1) controlled sugar feeding rate to achieve a pre-decided growth pattern by controlling the biomass growth rate at one preset value during growth phase and at another preset value during production phase by supplying a readily metabolizable sugar (like glucose); (2) constant sugar feeding rate to reproduce a pre-decided growth pattern; (3) ramp increase or decrease (or exponential increase or decrease) sugar feeding rate to maintain a constant sugar concentration in the system during production phase. Since the simulator is based on this mechanistic model that is valid for a specific parameter range, no further sensitivity analysis is performed. Users are advised to conduct sensitivity studies if the intent is to use large deviations from nominal conditions.

The unstructured model developed here may serve as a valuable tool for not only understanding the effects of a variety of operational variables on system dynamics but also for utilizing it for research and educational activities in modeling, monitoring, optimization and control.

## 2. Mathematical model of penicillin fermentation

In this work, the mechanistic model of Bajpai and Reuss (1980), which was shown to give a good agreement with the experimental results of Pirt and Righoletto (1967), was utilized as the starting point for model development. The original model has been extended by including additional input variables such as agitation power and aeration rate. Functional relationships among the process variables are summarized in Table 1 and all inputs and outputs are listed in Fig. 1. A variety of mathematical representations has been suggested for describing certain biological behaviors by researchers referenced earlier in the text and others. We used the representations by Bajpai and Reuss (1980) but readers are cautioned that several other representations

Table 1
Functional relationship among the process variables

| Model structure |
|---|
| $X = f(X, S, C_L, H, T)$ |
| $S = f(X, S, C_L, H, T)$ |
| $C_L = f(X, S, C_L, H, T)$ |
| $P = f(X, S, C_L, H, T, P)$ |
| $CO_2 = f(X, H, T)$ |
| $H = f(X, H, T)$ |

$X$, biomass concentration; $S$, substrate concentration; $C_L$, dissolved oxygen concentration; $P$, penicillin concentration; $CO_2$, carbon dioxide concentration; $H$, hydrogen ion concentration for pH ($[H^+]$); $T$, temperature.

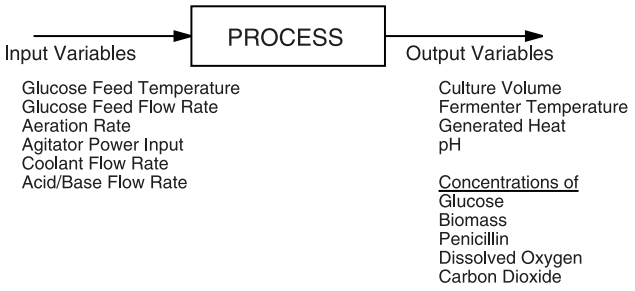


Fig. 1. Process input/output structure.

may also be used to describe the penicillin fermentation process.

### 2.1. Biomass growth

Experimental findings suggest a high degree of dependence of biomass growth on both the carbon source (glucose) and oxygen as substrates (Bajpai & Reuss, 1980). The biomass growth is also known to be inhibited by high amounts of biomass itself in penicillin fermentation. The dependence of specific growth rate on carbon and oxygen substrates was assumed to follow Contois kinetics (Bajpai & Reuss) to consider the biomass inhibition. The biomass growth has been described as:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \mu X - \frac{X}{V}\frac{\mathrm{d}V}{\mathrm{d}t} \tag{1}$$

where the specific growth rate $\mu$ was:

$$\mu = \mu_x \frac{S}{(K_x X + S)} \frac{C_L}{(K_{ox} X + C_L)} \tag{2}$$

in the original model (Bajpai & Reuss, 1980). The variables and parameters used are defined in Tables 1 and 2. Environmental variables such as pH and temperature play an important role on the quality and quantity of the final product. In order to take these effects into account, biomass formation has been related to these variables by introducing their effects in the specific growth rate expression to give:

$$\mu = \left[\frac{\mu_x}{1 + [K_1/[\mathrm{H}^+]] + [[\mathrm{H}^+]/K_2]}\right] \frac{S}{K_x X + S} \times \frac{C_L}{K_{ox} X + C_L} \left[k_g \exp\left(-\frac{E_g}{\mathrm{RT}}\right)\right] - \left[k_d \exp\left(-\frac{E_d}{\mathrm{RT}}\right)\right]. \tag{3}$$

This would in turn affect the utilization of substrate and the production of penicillin. Direct effects of pH and temperature on penicillin production are not considered due to the complex nature of the phenomenon, and unavailability of the experimental data.

### 2.2. Effect of pH

A typical inhibition term that includes hydrogen ion concentration $[\mathrm{H}^+]$ is introduced into the specific growth rate expression. Although the dependence of cell activity on pH cannot be explained possibly by this simple model, it is found that it gives an adequate fit for many microorganisms (Nielsen & Villadsen, 1994). The additional term is in the form:

$$\mu \propto f\left[\frac{\mu_x}{1 + [K_1/[\mathrm{H}^+]] + [[\mathrm{H}^+]/K_2]}\right] \tag{4}$$

Here, the values of $K_1$ and $K_2$ are chosen to be in the range of their typical values in the literature (Nielsen & Villadsen, 1994; Shuler & Kargi, 2002). It is a common practice to keep the pH constant during penicillin fermentation by adding $NH_4OH$ (Atkinson & Mavituna, 1991; Mou & Cooney, 1983). Since the pH of the culture medium has a tendency towards acidity, as the concentration of biomass increases, the amount of $NH_4OH$ added into the culture medium also increases. Based on this observation, the hydrogen ion concentration $[\mathrm{H}^+]$ is related to biomass formation as:

$$\frac{\mathrm{d}[\mathrm{H}^+]}{\mathrm{d}t} = \gamma\left(\mu X - \frac{FX}{V}\right) + \left[\frac{-B + \sqrt{(B^2 + 4 \times 10^{-14})}}{2} - [\mathrm{H}^+]\right]\frac{1}{\Delta t} \tag{5}$$

where $B$ is defined as:

$$B = \frac{[10^{-14}/[\mathrm{H}^+] - [\mathrm{H}^+]]V - C_{a/b}(F_a + F_b)\Delta t}{V + (F_a + F_b)\Delta t}. \tag{6}$$

$F_a$ and $F_b$ represent acid and base flow rates in l/h, respectively, where, the concentrations in both solutions are assumed equal as $C_{a/b} = 3$ M. The contribution of $[\mathrm{H}^+]$ change due to penicillin or any other metabolite concentration variation is not included in Eq. (5) due to lack of experimental data of this nature.

Under pH control, the hydrogen ion concentration is calculated by taking the dissociation of water and acid/base into account as well as the hydrogen production from biomass. The proportionality constant $\gamma$, is estimated as $10^{-5}$ mol $[\mathrm{H}^+]$/g biomass, based on the experimental data of Mou and Cooney (1983), which suggested a relationship between the biomass concentration and the hydrogen ion production by growth.

### 2.3. pH Control

The pH was kept constant at a value of 5.0 in order to simulate the observed behavior of penicillin production

Table 2
Initial conditions, kinetic and controller parameters for nominal operation

| Time $t$(h) | Value |
|---|---|
| *Initial conditions* | |
| Substrate concentration: $S$ (g/l) | 15 |
| Dissolved oxygen concentration: $C_L$ ($=C_L^*$ at saturation) (g/l) | 1.16 |
| Biomass concentration: $X$ (g/l) | 0.1 |
| Penicillin concentration: $P$ (g/l) | 0 |
| Culture volume: $V$ (l) | 100 |
| Carbon dioxide concentration: $CO_2$ (mmol/l) | 0.5 |
| Hydrogen ion concentration: $[H^+]$ (mol/l) | $10^{-5.1}$ |
| Temperature: $T$ (K) | 297 |
| Heat generation: $Q_{rxn}$ (cal) | 0 |
| *Kinetic parameters and variables* | |
| Feed substrate concentration: $s_f$ (g/l) | 600 |
| Feed flow rate of substrate: $F$ (l/h) | |
| Feed temperature of substrate: $T_f$ (K) | 298 |
| Yield constant: $Y_{x/s}$ (g biomass/g glucose) | 0.45 |
| Yield constant: $Y_{x/o}$ (g biomass/g oxygen) | 0.04 |
| Yield constant: $Y_{p/s}$ (g penicillin/g glucose) | 0.90 |
| Yield constant: $Y_{p/o}$ (g penicillin/g oxygen) | 0.20 |
| Constant: $K_1$ (mol/l) | $10^{-10}$ |
| Constant: $K_2$ (mol/l) | $7\times10^{-5}$ |
| Maintenance coefficient on substrate: $m_x$ (per h) | 0.014 |
| Maintenance coefficient on oxygen: $m_o$ (per h) | 0.467 |
| Constant relating $CO_2$ to growth: $\alpha_1$ (mmol $CO_2$/g biomass) | 0.143 |
| Constant relating $CO_2$ to maintenance energy: $\alpha_2$ (mmol $CO_2$/g biomass h) | $4\times10^{-7}$ |
| Constant relating $CO_2$ to penicillin production $\alpha_3$ (mmol $CO_2$/l h) | $10^{-4}$ |
| Maximum specific growth rate: $\mu_x$ (per h) | 0.092 |
| Contois saturation constant: $K_x$ (g/l) | 0.15 |
| Oxygen limitation constant: $K_{ox}$, $K_{op}$ (no limitation) | 0 |
| Oxygen limitation constant: $K_{ox}$, $K_{op}$ (with limitation) | $2\times10^{-2}$, $5\times10^{-4}$ |
| Specific rate of penicillin production: $\mu_p$ (per h) | 0.005 |
| Inhibition constant: $K_p$ (g/l) | 0.0002 |
| Inhibition constant for product formation: $K_I$ (g/l) | 0.10 |
| Constant: $p$ | 3 |
| Penicillin hydrolysis rate constant: $K$ (per h) | 0.04 |
| Arrhenius constant for growth: $k_g$ | $7\times10^{3}$ |
| Activation energy for growth: $E_g$ (cal/mol) | 5100 |
| Arrhenius constant for cell death: $k_d$ | $10^{33}$ |
| Activation energy for cell death: $E_d$ (cal/mol) | 50 000 |
| Density × heat capacity of medium: $\rho$ $C_p$ (per l °C) | 1/1500 |
| Density × heat capacity of cooling liquid: $\rho_c$ $C_{pc}$ (per l °C) | 1/2000 |
| Yield of heat generation: $r_{q_1}$ (cal/g biomass) | 60 |
| Constant in heat generation: $r_{q_2}$ (cal/g biomass h) | $1.6783\times10^{-4}$ |
| Heat transfer coefficient of cooling/heating liquid: $a$ (cal/h °C) | 1000 |
| Cooling water flow rate: $F_c$ (l/h) | |
| Constant: $b$ | 0.60 |
| Constants in $K_{la}$: $\alpha$, $\beta$ | 70, 0.4 |
| Constant in $F_{loss}$: $\lambda$ (per h) | $2.5\times10^{-4}$ |
| Proportionality constant: $\gamma$ (mol $[H^+]$/g biomass) | $10^{-5}$ |
| *Controller parameters (PID)* | |
| pH: (base) $K_c$, $\tau_I$: (h), $\tau_d$: (h) | $8\times10^{-4}$, 4.2, 0.2625 |
| pH: (acid) $K_c$, $\tau_I$: (h), $\tau_d$: (h) | $1\times10^{-4}$, 8.4, 0.125 |
| Temperature: (cooling) $K_c$, $\tau_I$: (h), $\tau_d$: (h) | 70, 0.5, 1.6 |
| Temperature: (heating) $K_c$, $\tau_I$: (h), $\tau_d$: (h) | 5, 0.8, 0.05 |

by utilizing an on/off or a proportional-integral-derivative (PID) controller. The pH is regulated by adding highly concentrated (3 M) acid or base solution when necessary. Two PID controllers are used to manipulate the acid and base control values. The PID controllers were tuned for a certain range of initial conditions considered to be the normal operation (Table 2). The maximum allowable acid and base additions were set to 0.01 and 100 ml/h, respectively. Fig. 2 shows the pH profile of a normal operation for different PID controller settings and for an on/off controller as well as the acid/base flow rates used in both cases. The initial pH is chosen arbitrarily as 5.1 for this particular case. Due to the acid addition rate limitation, the pH reached its desired value in almost 8 h of operation, then stayed within its control limits. A set point gap of 0.05 that can be adjusted by the users prior to simulation is defined for acid flow rate controller to avoid excessive acid additions. Acid solution is only added if the pH exceeds its set point value by 0.05. Acid solution is needed until pH drops below 5.05 in the case of initial pH value 5.1 in both on/off and PID controllers (Fig. 2b and c).

### 2.4. Effect of temperature

The influence of temperature on the specific growth rate of a microorganism shows an increasing tendency with an increase in temperature up to a certain value which is microorganism specific and a rapid decrease is observed beyond this value. This decrease might be treated as a death rate (Shuler & Kargi, 2002). Here, we have introduced the effect of temperature on the specific growth rate as an Arrhenius type of kinetics:

$$\mu \propto f\left\{\left[k_g \exp\left(-\frac{E_g}{RT}\right)\right]-\left[k_d \exp\left(-\frac{E_d}{RT}\right)\right]\right\} \quad (7)$$

$k_g$ and $E_g$ are the constant and activation energy for growth, while $k_d$ and $E_d$ are the constant and activation energy for death, respectively. Typical values for these parameters were taken from the literature (Shuler & Kargi, 2002). An adjustment has been made so that an increase in temperature enhanced the biomass formation up to 35 °C. This was followed by a rapid decrease in

biomass concentration as the temperature increased further as is the case in penicillin production.

### 2.5. Temperature control

The temperature of the culture medium was kept constant at 25 °C in accordance with literature (Atkinson & Mavituna, 1991; Mou & Cooney, 1983). A PID controller was designed to control the temperature of the culture by manipulating heating/cooling water flow rate (Marlin, 1995). Fig. 3 presents the temperature profiles under normal operation with different PID settings.

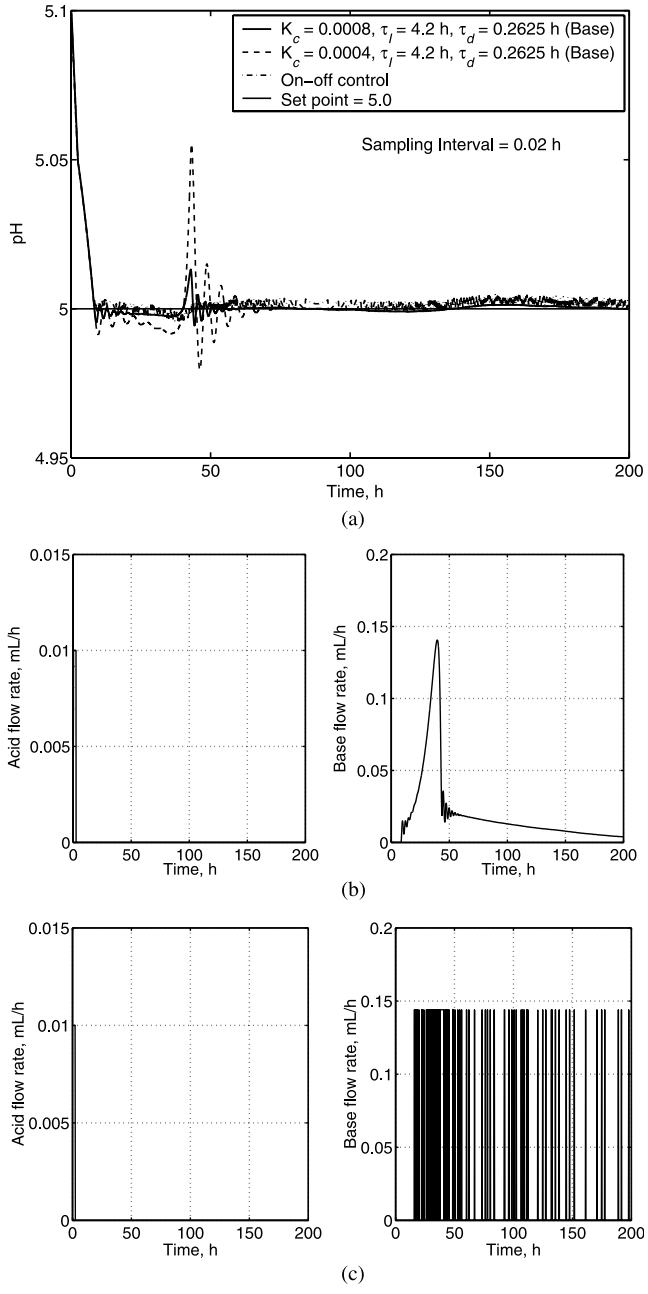


Fig. 2. (a) pH Profile of culture brothe under controlled conditions (pH set point is 5.0), In all cases, acid controller PID settings are $K_c = 1 \times 10^{-4}$, $\tau_I = 8.4$ h and $\tau_d = 0.125$ h. Acid and base flow rates used during the fermentation by (b) acid and base PID controllers (base controller PID settings are $K_c = 8 \times 10^{-4}$, $\tau_I = 4.2$ h and $\tau_d = 0.2625$ h), (c) acid and base on-off controller (for visual clarification data were plotted on every ten sampling interval).

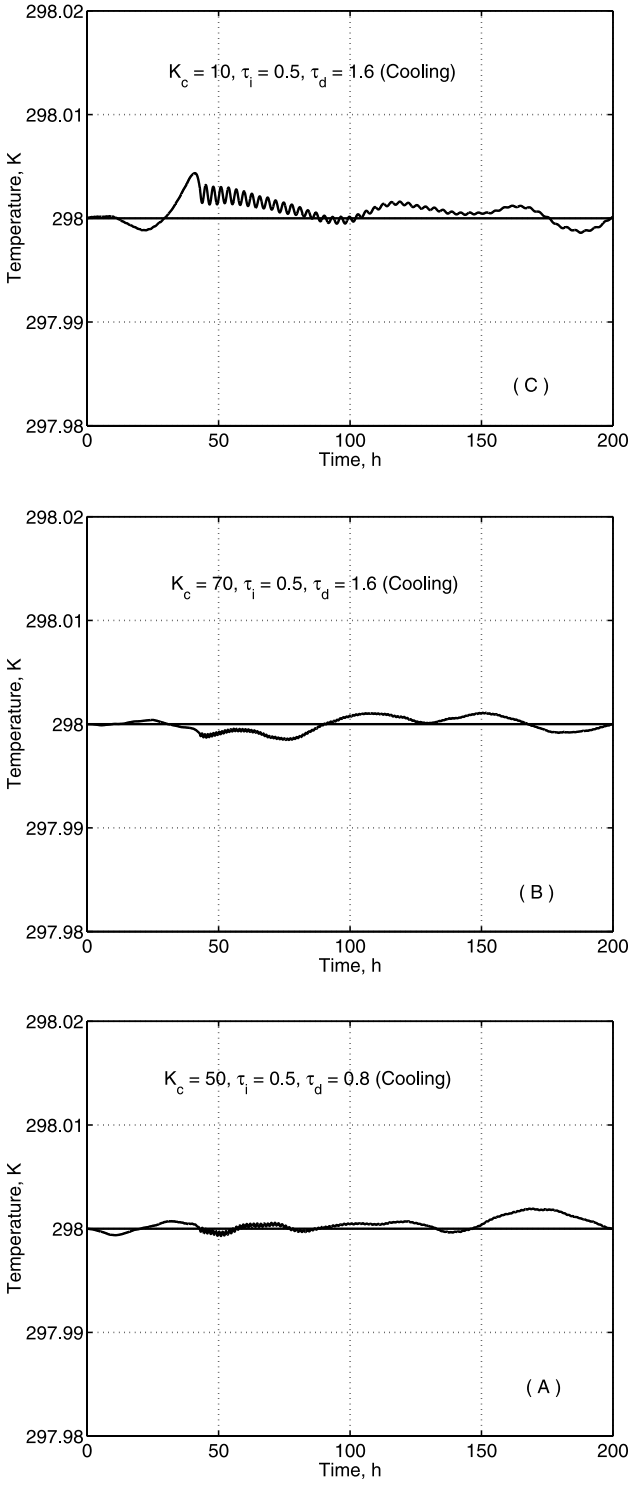


Fig. 3. Temperature control profiles under different PID controller settings. In all cases, the sampling interval is 0.02 h and the PID settings for heating are $K_c = 5$, $\tau_I = 0.8$ h and $\tau_d = 0.05$ h.

The velocity form of the digital PID algorithm is used in both pH and temperature controllers (Marlin, 1995).

$$\Delta MV_N = K_c\left(E_N - E_{N-1} + \frac{\Delta t}{\tau_I}E_N - \frac{\tau_d}{\Delta t} \times (CV_N - 2CV_{N-1} + CV_{N-2})\right)MV_N = MV_{N-1} + \Delta MV_N \quad (8)$$

where $K_c$ represents the proportional gain, $\tau_I$ integral constant, $\tau_d$ derivative constant, and $CV_N$, $SP_N$, and $MV_N$ denote the current values of the controlled variable, set point, and controller output at the current sample $N$, respectively. The current value of error is defined as $E_N = SP_N - CV_N$.

### 2.6. Penicillin production

The production of penicillin is described by non-growth associated product formation kinetics. The hydrolysis of penicillin is also included in the rate expression (Bajpai & Reuss, 1980):

$$\frac{dP}{dt} = \mu_{pp}X - KP - \frac{P}{V}\frac{dV}{dt} \quad (9)$$

where, $\mu_{pp}$ is the specific penicillin production rate defined as:

$$\mu_{pp} = \mu_p \frac{S}{(K_p + S + S^2/K_I)} \frac{C_L^p}{(K_{op}X + C_L^p)} \quad (10)$$

Substrate inhibition kinetics for penicillin production was originally proposed by Bajpai and Reuss (1980) that successfully represented the observed behavior. They commented that the proposed mechanism should not be considered to throw any light upon the nature of phenomena involved. Others point out that industrial strains of penicillin production are tolerant to high levels of glucose and question the use of substrate inhibition terms in Eq. (10). Large quantities of substrate results in only little improvement of penicillin production.

### 2.7. Substrate utilization

The utilization of substrate is assumed to be caused by biomass growth and product formation with constant yields and maintenance requirements of the microorganism as suggested by Bajpai and Reuss (1980).

Glucose:

$$\frac{dS}{dt} = -\frac{\mu}{Y_{x/s}}X - \frac{\mu_{pp}}{Y_{p/s}}X - m_x X + \frac{Fs_f}{V} - \frac{S}{V}\frac{dV}{dt} \quad (11)$$

Dissolved oxygen:

$$\frac{dC_L}{dt} = -\frac{\mu}{Y_{x/o}}X - \frac{\mu_{pp}}{Y_{p/o}}X - m_o X + K_{la}(C_L^* - C_L) - \frac{C_L}{V} \times \frac{dV}{dt} \quad (12)$$

In the original model of Bajpai and Reuss, the overall mass transfer coefficient $K_{la}$ is constant (Bajpai & Reuss, 1980). Here we have assumed $K_{la}$ to be a function of agitation power input $P_w$ and flow rate of oxygen $f_g$ as suggested by Bailey and Ollis (1986).

$$K_{la} = \alpha\sqrt{f_g}\left(\frac{P_w}{V}\right)^{\beta}. \quad (13)$$

The values of $\alpha$ and $\beta$ are assigned so that the dependence of penicillin concentration on $K_{la}$ showed a very similar behavior to the predictions of Bajpai and Reuss (1980). This has been discussed in detail in Section 4.

### 2.8. Volume change

The fed-batch process operation causes a volume change in the fermentor. This is calculated by:

$$\frac{dV}{dt} = F + F_{a/b} - F_{loss} \quad (14)$$

In order to consider the effect of acid/base addition on the total volume change of the culture broth, we have included the second term, $F_{a/b}$. We have also included the $F_{loss}$ term to account for evaporative loss during fermentation. The loss in volume due to evaporation is in fact more significant in industrial fermentations than the base addition term. Normally the air entering the fermentor is fairly dry and it is at about 90–100% relative humidity after bubbling through the broth. Typically, 10–20% of the total broth can be lost due to evaporation during 1 week of fermentation, the actual amount depending on the temperature of the fermentation. Here, we have taken $F_{loss}$ to be a function of temperature and culture volume $V$ of the fermentation broth:

$$F_{loss} = V\lambda(e^{5((T-T_0)/T_v - T_0)} - 1) \quad (15)$$

where $T_0$ and $T_v$ are the freezing and boiling temperatures of the culture medium that were assumed to have the same properties as water, respectively. We have assumed that the evaporation rate will tend to infinity at the boiling point, and for engineering purposes the exponent 5 is large enough to represent this. $\lambda$ is arranged to give an evaporation rate of $2.5 \times 10^{-4}$ l/h at the operation temperature (25 °C). This expression suggests including an evaporative loss term in volume due to temperature rather than proposing a mechanism. Since we do not base our $F_{loss}$ term on experimental

findings, this should be treated as a *heuristic* since as a general tendency, the temperature increase favors evaporative loss. A more accurate relationship can be developed by carrying out a set of experiments at different temperatures and measuring the corresponding humidity of the inlet/exit gas and the volume of the culture broth with respect to time.

### 2.9. Heat of reaction

Neglecting all other sources of heat generation except that caused by microbial reactions, the volumetric heat production rate is given as:

$$\frac{dQ_{rxn}}{dt} = r_{q_1}\frac{dX}{dt}V + r_{q_2}XV \tag{16}$$

where $r_{q_1}$ is assumed to be constant and might be treated as a yield coefficient (Nielsen & Villadsen, 1994). During the product synthesis phase, when the rate of biomass formation becomes very small there is still significant heat generation from metabolic maintenance activities. Therefore, we have included the second term in Eq. (16) to account for the heat production during maintenance. Because the heat generation and $CO_2$ evolution show similar profiles, their production rate due to growth ($dX/dt$) and biomass ($X$) should have the same ratio as a first approximation. Based on this observation, $r_{q_2}$ is calculated and tabulated in Table 2. The energy balance is written based on a coiled type heat exchanger which is suitable for a laboratory scale fermentor (Nielsen, 1997):

$$\frac{dT}{dt} = \frac{F}{s_f}(T_f - T) + \frac{1}{V\rho c_p} \times \left[Q_{rxn} - \frac{aF_c^{b+1}}{F_c + (aF_c^b/2\rho_c c_{pc})}\right] \tag{17}$$

A lab-scale fermentor was assumed in deciding to use a cooling coil, building the model, and developing the related equations. For industrial scale fermentors, the heat transfer mechanism should be modified to a jacketed fermentor with baffles and appropriate equations must be substituted.

### 2.10. $CO_2$ evolution

The introduction of variables which are easy to measure yet important in terms of their information content has been very helpful in predicting other important process variables. One such variable is $CO_2$ from which biomass may be predicted with high accuracy. In this work, $CO_2$ evolution is assumed to be due to growth, penicillin biosynthesis and maintenance requirements as suggested by Montague, Morris, Wright, Aynsley and Ward (1986). The $CO_2$ evolution is:

$$\frac{dCO_2}{dt} = \alpha_1\frac{dX}{dt} + \alpha_2 X + \alpha_3 \tag{18}$$

Here, the values of $\alpha_1$, $\alpha_2$ and $\alpha_3$ are chosen to give $CO_2$ profiles similar to the predictions of Montague et al. (1986). $CO_2$ evolution is nearly the same as oxygen demand for penicillin production using glucose as a substrate and $CO_2$ evolution trend levels off after the fed-batch switch as expected.

The extended model we have developed consists of differential Eqs. (1), (3), (9)–(18) that are solved simultaneously. In fermentation processes, particularly in penicillin production, parameter values are closely related to medium composition, feed composition and the choice of the strain. It is essential to assess the adequacy of model equations for their validity and appropriateness of the kinetic and operation parameters to use them with different medium and strain conditions.

## 3. Simulation software

The simulator was originally developed in MATLAB 6.0 environment and modularized functions were compiled into ANSI C codes and these C modules are further compiled to produce an executable stand-alone application file. A tutorial file suggests ranges for inputs and parameters. The simulation package is available at http://www.chee.iit.edu/ ~control/simulation.html. There are no hard restrictions on the input variables and the kinetic parameters in this simulator although ranges of various inputs and parameters are suggested. Hence, there is no guarantee that physically meaningful outputs will be obtained depending on the choice of the initial conditions and parameters. For this reason, one has to be careful about the interpretation of the results. The nominal operating conditions, kinetic and controller parameters are summarized in Table 2. During the simulation, the sampling time was chosen to be 0.02 h as default. Simulations are run under closed-loop control of pH and temperature while glucose addition is performed open-loop. In bioprocesses, most of the important process variables such as biomass and penicillin concentrations are analyzed off-line by the quality analysis laboratory resulting in a lag in process measurements. For that reason, the sampling time should be adjusted accordingly to compensate for that lag. There are new on-line measurement devices that are being developed to overcome this difficulty. A sampling time of 0.02 h is reasonable for practical applications for readily measurable variables. The simulator gives the user the flexibility of changing the sampling time for using state estimators based on frequently measured variables. In order to avoid any loss in the controller performance all the simulations are run at 0.02 h of

sampling time, but users have the flexibility of collecting and/or plotting the simulated results at their choice of sampling time. Although a sampling time of 0.02 h is reasonable for certain variables such as off-gas analysis, pH and temperature, it is not reasonable for some other variables such as cell mass and penicillin concentrations since it is difficult to measure them for practical purposes. In industrial scale fermentations such variables are usually measured off-line via HPLC every 8–10 h.

The user can adjust process operating conditions to introduce certain disturbances to input variables.

The user-friendly nature of the package enables the user to conduct studies such as:

1) Empirical model development.
2) Development and testing of model order reduction algorithms.
3) Process database construction to develop statistical monitoring/control tools.
4) Process optimization and testing of various optimization algorithms.
5) Implementation of different controller designs.
6) Studying controller performance assessment.

The following capabilities of the simulator provides a flexible environment to conduct these studies:

1) Option to run the simulator in default initial conditions, kinetic and controller parameters.
2) User defined initial conditions, kinetic and controller parameters.
3) Controller type selection; on/off (pH), PID (pH, temperature).
4) Options to implement different operation policies; the presence/absence of oxygen limitation, batch/fed-batch switching.
5) Option to exclude the effects of environmental variables (pH and temperature).
6) Option to keep process history in ASCII format.

## 4. Results and discussion

We have developed an educational software for simulating penicillin production in a fed-batch fermentor. A detailed unstructured mathematical model is proposed in order to include many realistic input variables. Fluctuations are introduced as pseudo-random binary signals to input variables. White noise is added to $CO_2$ and dissolved oxygen profiles to account for the sensor sensitivities. Fig. 4 represents the profiles of output variables under nominal operating conditions where a constant glucose feed is used during the fed-batch operation. A small fluctuation is added to glucose feed rate (Fig. 4c) as smoothed pseudo-random binary signal (PRBS) to mimic the real process environment.

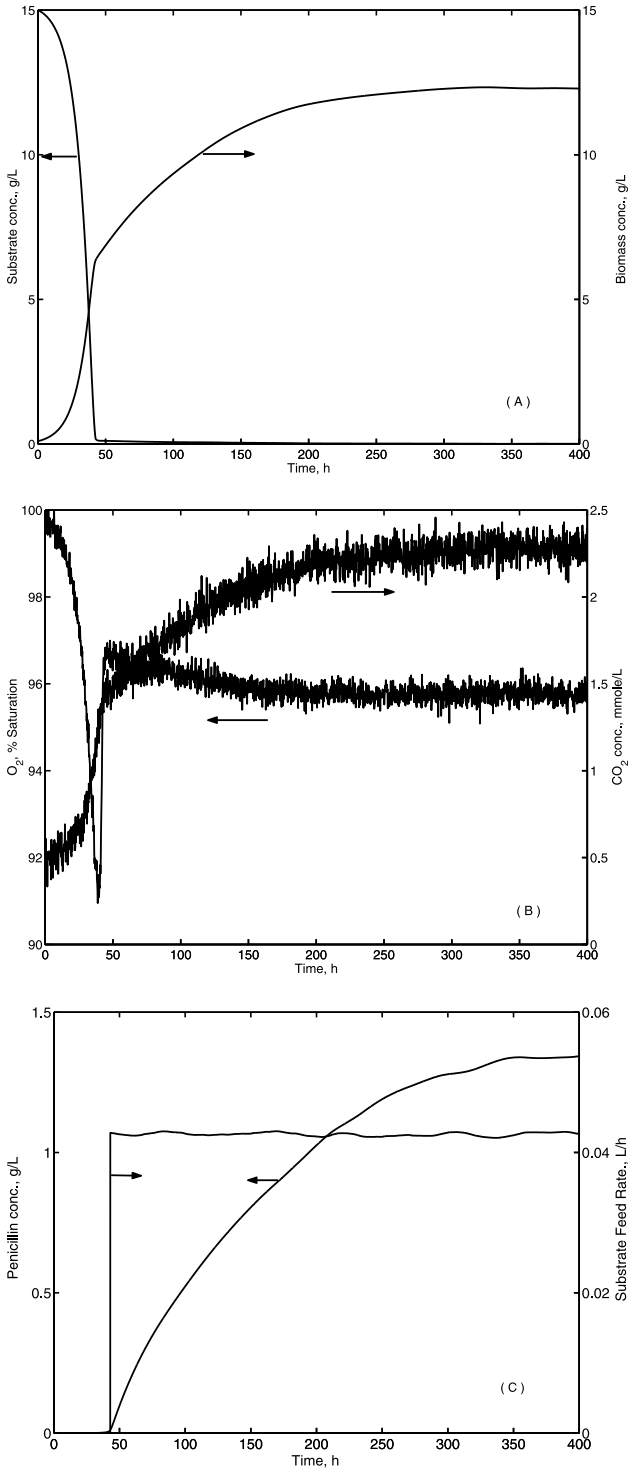


Fig. 4. (A) Time courses of glucose and biomass concentrations; (B) dissolved oxygen and carbon dioxide concentrations; (C) penicillin concentration and substrate feedback at nominal conditions of a batch followed by a fed-batch operation for an initial substrate and biomass concentrations of 15 g/l and 0.1 g/l, respectively. pH and temperature are controlled via PID controllers at 5.0 and 25 °C, respectively.

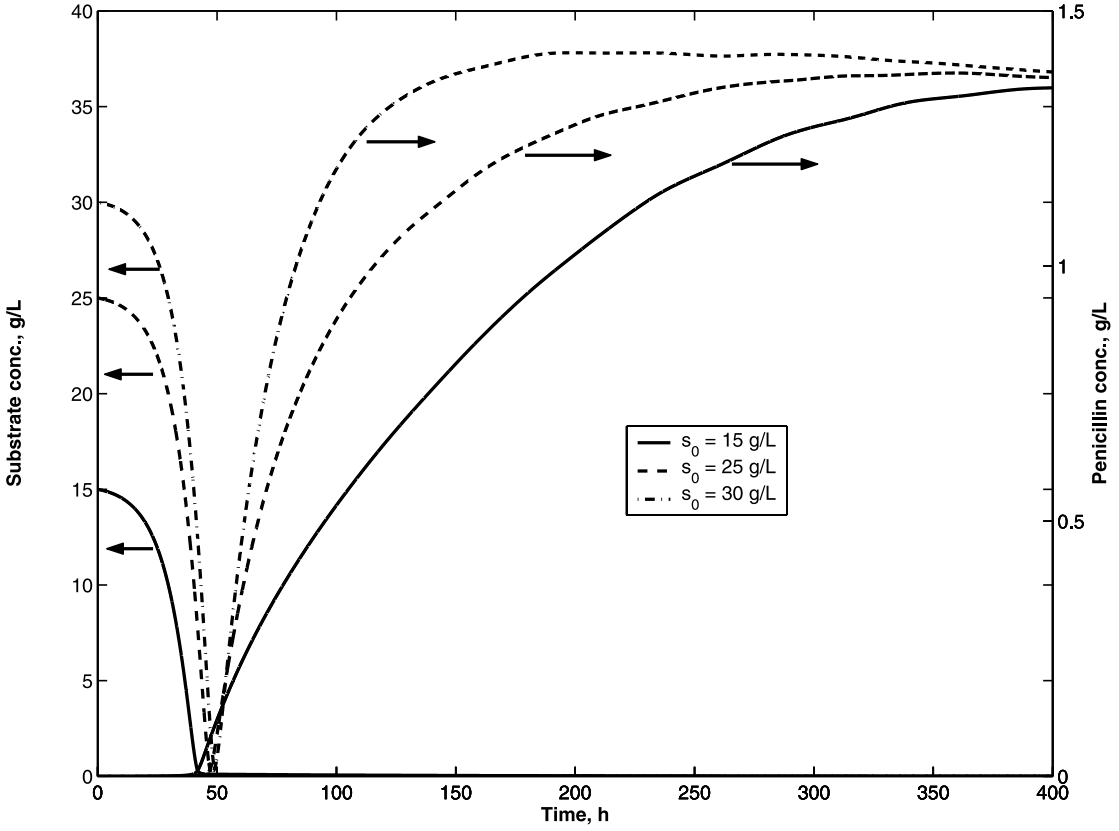


Fig. 5. Glucose and penicillin concentrations at initial glucose concentrations of 15, 25, 30 g/l.

Simulations have been carried out to check the performance of the simulator. In all runs, a batch culture has been followed by a fed-batch operation by the depletion of the carbon source (glucose). This has been done by assigning a threshold value to glucose concentration which was chosen as 0.3 g/l. The system

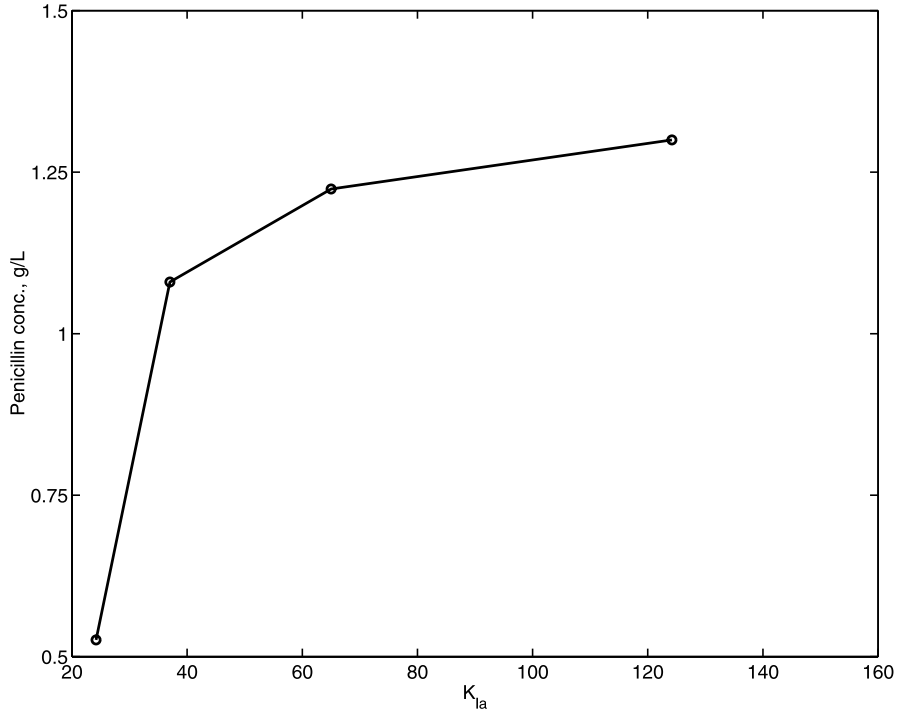


Fig. 6. Dependence of penicillin concentration on overall mass transfer coefficient, $K_{la}$ under oxygen limitation conditions (oxygen saturation $\leq$ 50%).

Table 3
A summary of the faults introduced at different stages of fermentation

| Fault number | Fault type | Occurrence time (h) |
|---|---|---|
| 1 | pH Controller failure | 10 |
| 2 | 95% step decrease in agitation power (under oxygen limitation) | 30 |
| 3 | 30% step decrease in substrate feed rate | 60 |

switches itself to the fed-batch mode of operation when the level of glucose concentration reaches this threshold value.

An approach similar to Bajpai and Reuss (1980) has been followed to test the validity of the model. The predictions of the model under different conditions are compared with experimental data of Pirt and Righoletto (1967) and the simulation results of Bajpai and Reuss (1980). Note that most of the parameters are functions of the strain, the nature of substrate and the environmental conditions like pH and temperature. The additional terms that were introduced increased the stiffness of the ordinary differential equations. For that reason, some of the parameter values are readjusted. All the parameter values are listed in Table 2.

Fig. 5 shows the simulation results of penicillin fermentation at different initial carbon source (glucose) concentrations under normal operating conditions. Penicillin production showed an increasing trend with an increase in the initial glucose concentration then levelled off. This was attributed to the inhibition of biomass due to high concentration of substrate. In another set of runs, $K_{la}$ was assigned different values by changing agitator power input and flow rate of oxygen under oxygen limitation (oxygen saturation $\leq$ 50%). Penicillin concentration increased with increasing $K_{la}$ values, then it levelled off indicating a high degree of dependence of penicillin production on oxygen (Fig. 6). The simulator mimicked the biomass, penicillin, glucose and oxygen concentrations in a similar fashion to the predictions of Bajpai and Reuss (1980) which were also validated by the experimental results of Pirt and Righoletto (1967). All these findings supported the reliability and applicability of the proposed model.

To gain more insight on the model predictions, a series of disturbances were introduced at different stages of fermentation. Table 3 summarizes these disturbances and their time of occurrence. Fig. 7 represents the penicillin production under various process faults. Fault 1 shows the pH controller failure at 10 h of operation. That was implemented by turning the pH controller off in the process until the end of the fermentation. Since the process dynamics tends towards higher acidity under open loop conditions, and no base addition takes place in the absence of pH controller, this in turn affects the maximum rate of cell production (Eq. (4)) resulting in a decrease in total cell mass concentration which is associated with a decrease in penicillin concentration. Fault 2 is a substantial step decrease in agitator power input at 30 h of operation, and it affected mainly the dissolved oxygen concentration in the culture medium. As given by Eq. (13), agitation has a direct influence on the overall oxygen mass transfer coefficient, $K_{la}$. A decrease in the $K_{la}$ value resulted in a decrease in the

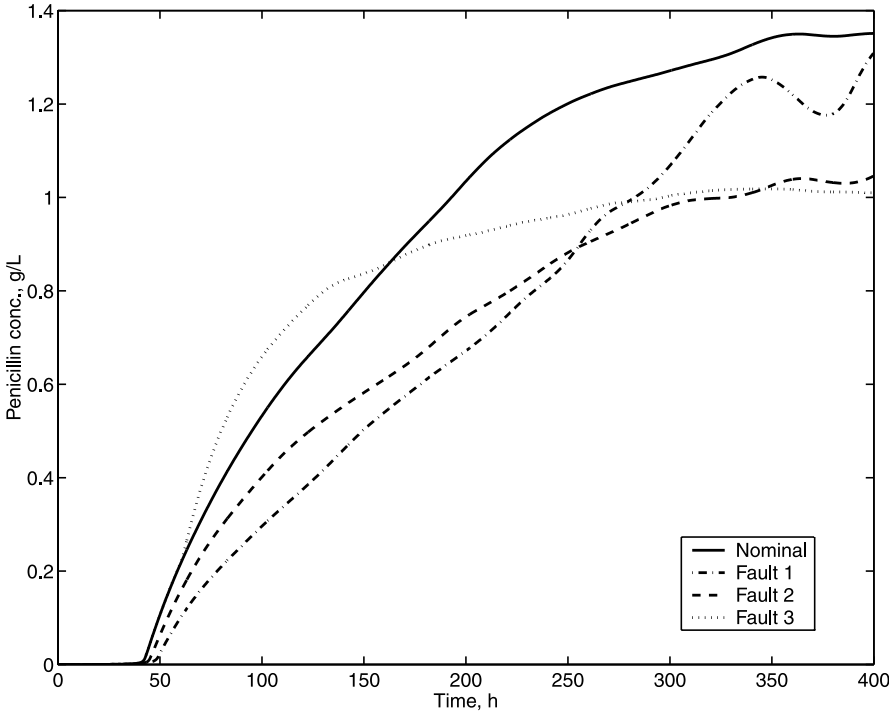


Fig. 7. Penicillin concentration at faulty cases.

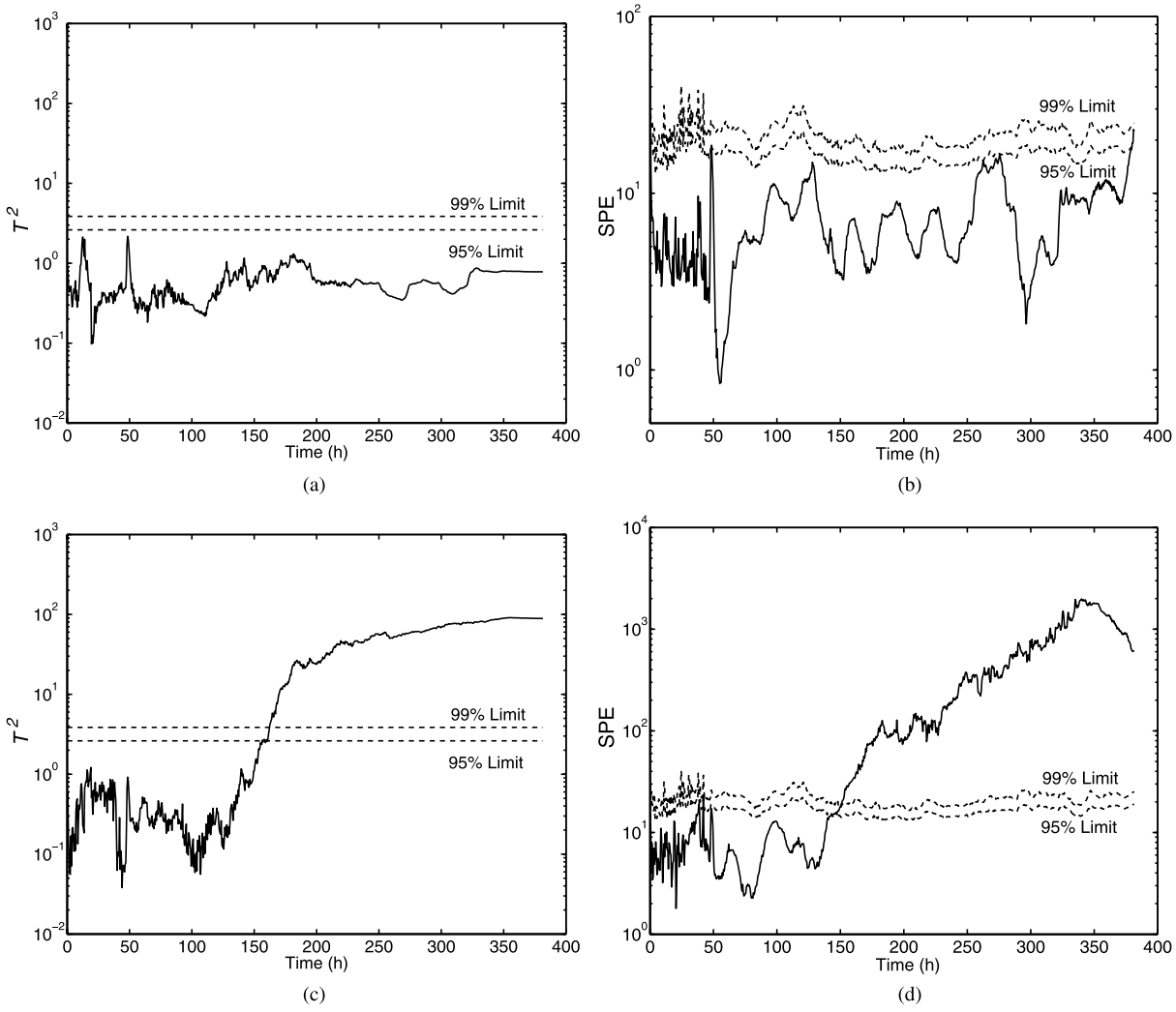


Fig. 8. Multivariate control charts monitoring new batches. (a) $T^2$; (b) SPE charts for a non-reference batch under normal operation; (c) $T^2$; (d) SPE charts for a faulty batch with a small drift in glucose feed rate.

dissolved oxygen level in the culture medium, consequently biomass growth and penicillin concentration lowered. This type of behavior has already been observed when we tested the simulator (Fig. 6). Penicillin concentration was again affected severely when Fault 3, a step change in substrate feed rate at 60 h of operation, has been introduced and retained until the end of fermentation. Glucose is the main carbon source to be fed during the fed-batch fermentation and a decrease in its feed results in reduction in penicillin production.

One of the potential uses of the simulator is to produce data to develop and evaluate statistical process performance monitoring techniques (Cinar & Undey, 1999; Undey et al., 2000). Multiway principal component analysis (MPCA) technique was utilized to construct a statistical process monitoring framework. MPCA is based on PCA (Wold, Geladi, Esbensen & Ohman, 1987). It is equivalent to performing ordinary PCA on a large two-dimensional matrix constructed by unfolding the three-way array. It has been applied to the monitoring of batch processes in recent years (Nomikos & MacGregor, 1994; Cinar & Undey, 1999; Undey et al., 2000). Statistical process monitoring based on MPCA can be implemented by graphical and numerical tools. Two types of statistics, the statistical distance $T^2$ and the principal components model residuals or squared prediction error (SPE) must be monitored. The SPE and $T^2$ charts indicate when the process goes out of control, but they do not provide information on the source causes of abnormal process operation. The diagnosis activity can be done by determining which process variables have contributed to inflate $T^2$ and SPE using contribution plots (Kourti & MacGregor, 1996; Miller, Swanson & Heckler, 1998).

In this study, a reference data set was produced by running the simulator repeatedly under normal operating conditions with small random variations. The reference data set (50 batches) served as a historical database and was used to model the normal behavior of

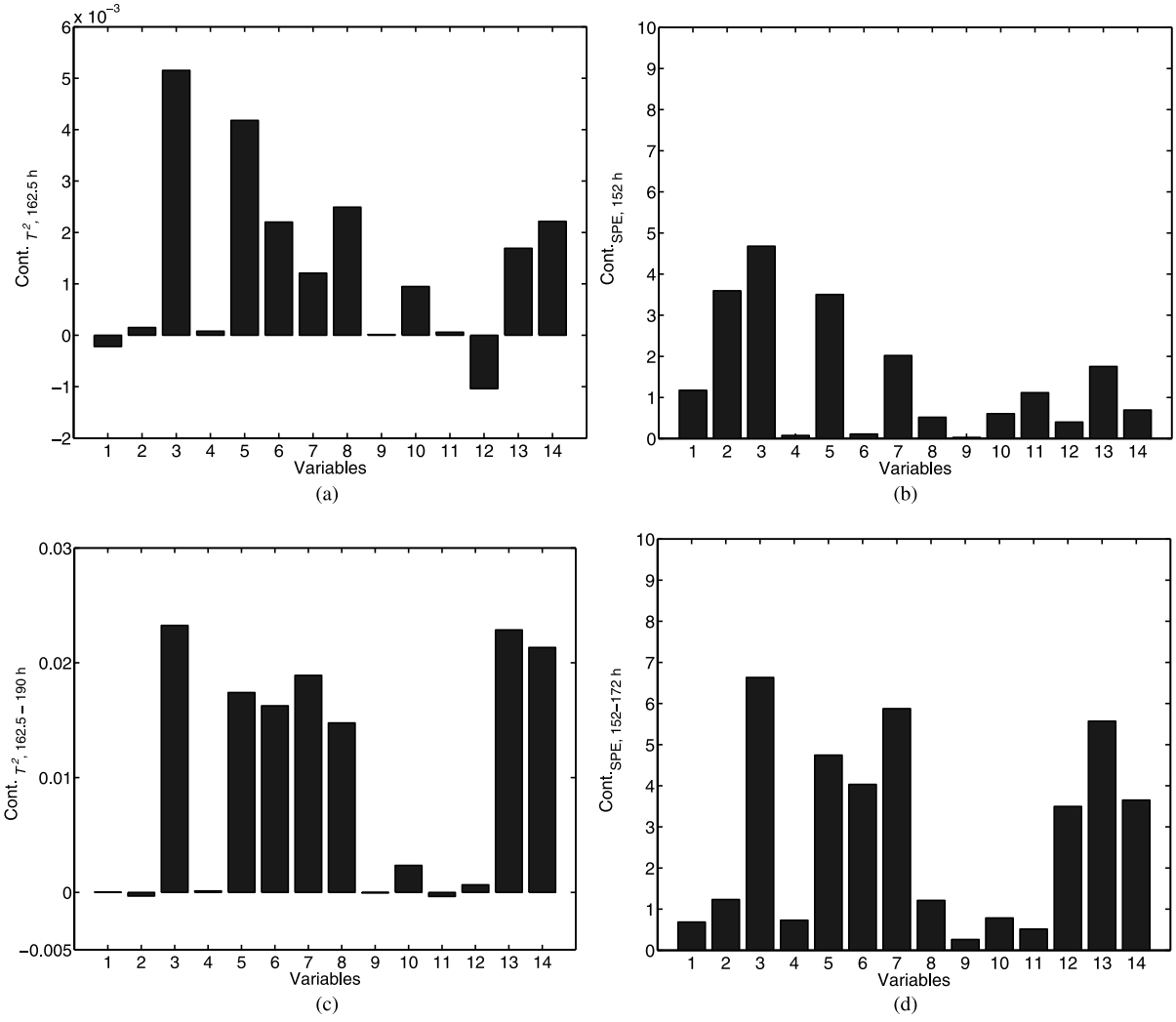


Fig. 9. Variable contribution to $T^2$, and SPE values for fault diagnostics in a faulty batch. Contributions to (a) $T^2$; and (b) SPE at the time of first out-of-control signal; average contributions to; (c) $T^2$; and (d) SPE after out-of-control situation is observed.

the process. Fig. 8a and b show the monitoring results of a non-reference batch run under normal operating conditions. All multivariate control charts indicate an in-control behavior for this new batch run. A small reduction was introduced to glucose feed rate (variable 3) in another run from the start of fed-batch operation to test the empirical model and monitoring techniques. SPE and Hotelling's $T^2$ charts are used to detect out-of-control situation (Fig. 8). Both $T^2$ (Fig. 8c) and SPE (Fig. 8d) charts have successfully detected the out-of-control situation on time. Since these charts are only able to detect abnormalities in the process, the contribution plots are used to diagnose the particular fault (Fig. 9). The contribution plots have shown which variable(s) (Table 4) was (were) responsible for the inflation on the SPE and $T^2$-values. By investigating the contribution plots of the faulty batch, variables 3 (glucose feed rate) and 5 (glucose concentration in the fermentor) were found to be the variables responsible for causing deviation from the normal behavior at the time of first out-of-control signal at 162.5 h on $T^2$ (Fig. 9a) and 152 h on SPE (Fig. 9b). The first out-of-control signal is observed at 228.5 h when only univariate charts

Table 4
Variable legends in Figs. 8 and 9

| Variable number | Definition |
|---|---|
| 1 | Aeration rate |
| 2 | Agitator power input |
| 3 | Substrate feed rate |
| 4 | Substrate feed temperature |
| 5 | Substrate concentration |
| 6 | Dissolved oxygen concentration |
| 7 | Biomass concentration |
| 8 | Penicillin concentration |
| 9 | Volume |
| 10 | Carbon dioxide concentration |
| 11 | Hydrogen ion concentration |
| 12 | Temperature |
| 13 | Generated heat |
| 14 | Cooling water flow rate |

are used. Variable contributions to $T^2$ and SPE over a period of time after the first out-of-control signal is observed on each chart (Fig. 9c and d), unveils the after-effects of the original fault. Contributions to inflated $T^2$ and SPE, averaged over time interval of 162.5 h and 190 h for $T^2$ (Fig. 9c), and 152 h and 172 h for SPE (Fig. 9d), reveal additional variables that are sequentially affected such as variable 6 (dissolved oxygen concentration), 7 (biomass concentration), 8 (penicillin concentration), 13 (heat generated) and 14 (cooling water flow rate). If fault detection were delayed, diagnosis would be more complicated since all these variables would indicate significant deviations from their expected values.

## 5. Conclusions

A detailed unstructured model is proposed for penicillin production in a batch/fed-batch process to extend a model reported earlier. The mathematical model contains additional input variables such as pH, temperature, aeration rate, agitation power, feed flow rate of substrate as well as output variables like $CO_2$ evolution and heat generation terms. With the introduction of pH and temperature terms to the model equations, it is possible to investigate the influences of such environmental variables on system dynamics. Multivariate statistical process monitoring and fault diagnosis techniques were applied to penicillin fermentation using the simulated data generated by the model developed.

The simulator developed for penicillin production is available at http://www.chee.iit.edu/ ~control/software.html and may serve as a tool for many academic and industrial applications. The user-friendly nature of the simulator provides flexibility to the user such as changing the parameters and initial conditions and introducing the faults that affect various input variables.

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