MODEL PREDICTIVE CONTROL ON FED-BATCH PENICILLIN
FERMENTATION PROCESS

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the requirement of the award of the degree of
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I declared that this thesis entitled “Model Predictive Control on Fed-Batch Penicillin Fermentation Process” is the result of my own researched excepted as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : _______________________
Name : CHEW LI MEI
Date : 20 APRIL 2009
To my beloved mother and father
ACKNOWLEDGEMENT

Praise is to God for His help and guidance that finally I able to complete this undergraduate project as one of the requirement of my degree study.

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ABSTRACT

In this research study the development of optimization strategies for a fed-batch penicillin fermentation process using model predictive controller was simulated using MATLAB 7.1 software. To facilitate the study, model predictive control (MPC) based on unstructured model for penicillin production in a fed-batch fermentor has been developed. A mathematical model of the system is derived based on published materials, the data is generated using PENSIM, dynamic response is analyzed, transfer function is developed and finally the MPC is implemented into the fermentation process. MPC offers an adaptive and optimizing control strategy which deals with multiple goals and constraints. The results of a study of the applicability of Model Predictive Control (MPC) in the process were obtainable. In order to obtain best optimization result for the fed-batch penicillin fermentation process, two optimization algorithms were selected. First, dynamic optimization using direct shooting method and second is implementation single step ahead Dynamic Matrix Control (DMC). Comparison of these two different approaches shows that DMC algorithm showed the best result with an optimization procedure.
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**LIST OF NOMENCLATURE**

- $a$ - Heat transfer coefficient of heating/cooling liquid (cal/h.$^\circ$C)
- $C_{\text{a/b}}$ - Acid or base concentration (molar)
- $C_L$ - Dissolved oxygen concentration (= $C_L^*$ at saturation) (g/l)
- CO$_2$ - Carbon dioxide concentration (mmol/l)
- $c_p$ - Heat capacity of medium (cal/g.$^\circ$C)
- $c_{pc}$ - Heat capacity of cooling liquid (cal/g.$^\circ$C)
- $E_d$ - Activation energy for cell death (cal/mol)
- $E_g$ - Activation energy for growth (cal/mol)
- $f_g$ - Oxygen flow rate (l/h)
- $F$ - Feed flow rate of substrate (l/h)
- $F_a$ - Acid flow rate (ml/h)
- $F_b$ - Base flow rate (ml/h)
- $F_c$ - Cooling water flow rate (l/h)
- $h$ - Specific enthalpy
- [H$^+$] - Hydrogen ion concentration (mol/l)
- $k_d$ - Arrhenius constant for cell death
- $k_g$ - Arrhenius constant for growth
- $K$ - Penicillin hydrolysis rate constant (h$^{-1}$)
- $K_1$ - Constant (mol/l)
- $K_2$ - Constant (mol/l)
- $K_1$ - Inhibition constant for product formation (g/l)
- $K_{\text{OP}}$ - Oxygen limitation constant
- $K_{\text{OX}}$ - Oxygen limitation constant
- $K_P$ - Inhibition constant (g/h)
- $m_o$ - Maintenance coefficient on oxygen (h$^{-1}$)
- $m_X$ - Maintenance coefficient on substrate (h$^{-1}$)
\[ P \] - Penicillin concentration (g/l)
\[ P_w \] - Agitation power input (W)
\[ q_s \] - Specific rate of substrate
\[ q_o \] - Specific oxygen uptake rate
\[ Q_o \] - Rate of oxygen uptake per volume of broth
\[ Q_{rxn} \] - Heat generation due to microbial metabolism (KJ/kg)
\[ Q_{ag} \] - Heat generation due to mechanical agitation (KJ/kg)
\[ Q_{gas} \] - Heat generation due to aeration power input (KJ/kg)
\[ Q_{exch} \] - Heat generation due to the surroundings and/or heat exchanger (KJ/kg)
\[ Q_{sen} \] - Rate of sensible enthalpy
\[ R \] - Gas constant (1.987 cal/(mol.K))
\[ r_{q1} \] - Yield of heat generation (cal/g biomass)
\[ r_{q2} \] - Constant in heat generation (cal/g biomass.h)
\[ s_f \] - Feed substrate concentration (g/l)
\[ S \] - Substrate concentration (g/l)
\[ T \] - Temperature (K)
\[ T_f \] - Feed temperature of substrate (K)
\[ V \] - Culture volume (l)
\[ X \] - Biomass concentration (g/l)
\[ Y_{P/O} \] - Yield constant (g penicillin/g oxygen)
\[ Y_{P/S} \] - Yield constant (g penicillin/g glucose)
\[ Y_{X/O} \] - Yield constant (g biomass/g oxygen)
\[ Y_{X/S} \] - Yield constant (g biomass/g glucose)
\[ \alpha \] - Constant in Kla
\[ \alpha_1 \] - Constant relating CO\textsubscript{2} to growth (mmol CO\textsubscript{2}/g biomass)
\[ \alpha_2 \] - Constant relating CO\textsubscript{2} to maintenance energy (mmol CO\textsubscript{2}/g biomass.h)
\[ \alpha_3 \] - Constant relating CO\textsubscript{2} to penicillin production (mmol CO\textsubscript{2}/l.h)
\[ B \] - Constant in Kla
\[ \gamma \] - Proportionality constant (mol [H\textsuperscript{+}]/g biomass)
\[ \mu_X \] - Maximum specific growth rate (h\textsuperscript{-1})
\[ \mu_P \] - Specific rate of penicillin production (h\textsuperscript{-1})
\[ \rho \] - Density of the culture medium (g/l)
\[ \rho_c \] - Density of the cooling liquid (g/l)
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CHAPTER 1

INTRODUCTION

1.1 Background Study

In recent years the performance requirements for processes in chemical and biotech processes have become increasingly difficult to satisfy. Modern plants have become more complicated to operate because of its trend toward complex and highly integrated processes. Therefore, dynamic model of the process was created with the intention that can be used in a computer simulation to evaluate alternative control strategies and to determine initial values of the controller settings.

There are many aspects that complicate the modeling of the bioprocesses. A fermentation process has both nonlinear and dynamic properties. The metabolic processes of the microorganisms are very complicated and cannot be modeled precisely. Because of these reasons, traditional modeling methods fail to model bioprocesses accurately. The modeling is further complicated because the fermentation runs are usually quite short and large differences exist between different runs.

Fermentations can be operated in batch, fed-batch or continuous reactors. In batch reactor all components, except gaseous substrates such as oxygen, pH-controlling substances and antifoaming agents, are placed in the reactor in the beginning of the fermentation. During process there is no input nor does output flow. In fed-batch process, nothing is removed from the reactor during the process, but one
substrate component is added in order to control the reaction rate by its concentration. There are both input and output flows in a continuous process, but the reaction volume is kept constant. As explained by Yuan et al. (1997), although continuous processes offer advantages such as higher productivity and ease of operation compared to batch processes, they retain certain disadvantages such as more severe impacts due to equipment failures, infection by other microorganisms, and spontaneous mutations in the strain. On the other hand, fed-batch modes are preferred since it provides better management of substrates and is able to avoid excessive substrate feed, which can inhibit microorganism growth. Since products are also withdrawn at the end of the batch, sterilized conditions can be maintained during process operation.

In this research study, penicillin production is considered due to its nonlinear dynamics and multistage nature as well as its industrial importance. The mechanistic model has been substantially improved by the inclusion of aeration rate, agitation power, feed flow rates of substrate and oxygen, carbon dioxide concentration, feed coolant and bioreactor temperatures, generated heat and the medium pH.
1.2 Problem Statement

Penicillin is produced by microorganism fermentation method. Until now, the production of penicillin still continues to attract research interest. This is because penicillin has significant benefit towards the commercial and therapeutic, and giving impact to the engineering field. Penicillin fermentation processes are complex bioprocesses of microorganism community growth, circulation and metabolism. Fermentation production involves high operating cost and energy consumption. Hence, in order to reduce penicillin production cost, increasing its yield and quality, penicillin fermentation production processes need to be optimized.

In fed batch fermentation penicillin process, inequality constraints do occur on input and output variable. Input constraints take place as a result of physical limitations on plant equipment such as pumps, control valves, and heat exchangers. Besides that, many output variables do not have set points. For these output, the control objectives is to maintain them between upper and lower limit instead of forcing them to set points. The inability to provide on-line measurement of fermentation variables such as biomass concentration has proved to be a significant obstacle for the implementation of advanced control and optimizations solutions in the fed batch fermentation penicillin process [Zhang and Lennox et al., 2004]. Therefore, Model Predictive Control (MPC) is used to solve the problem besides improving the simulation of penicillin production.

1.3 Objective

The main objective of this research study is to apply Model Predictive Control base on fed batch penicillin fermentation process using MATLAB 7.1 software.
1.4 Scope of Study

To achieve the objective, the following scope of research is proposed:

i. Identification of mathematical models for the fed-batch fermentation process
ii. Collecting data generation from fed batch fermentation process
iii. Analyzing the dynamic response of the fed batch fermentation process
iv. Development of transfer function based on dynamic response
v. Implementation of Model Predictive Control on fed batch fermentation process

1.5 Significant of Study

Model Predictive Control has given major impact on chemical industrial practice, with over 4500 applications worldwide. In these industries, MPC has become a method of choice for difficult multivariable control problem. MPC is an important advanced control technique for large multiple input-output with difference constraints on the inputs and/or output. If the model is accurate and representative of the process being considered, simulation studies will provide close guidance to what is supposed the optimum conditions for actual implementations. This will save not only time and efforts but also cost of operations.
Chapter 2

Literature Review

2.1 Penicillin

Penicillin is a group of Beta-lactam antibiotics used used to treat many different types of infections caused by a wide range of Gram-positive bacteria. “Penicillin” is also the informal name of a specific member of the penicillin group Penam Skeleton, which has the molecular formula R-C₉H₁₁N₂O₄S, where R is a variable side chain. Penicillin was the first naturally-occurring antibiotic discovered and the first to be used therapeutically. Its wide usage is a result of its lack of toxicity and irritancy. It works by interfering with the formation of the bacteria's cell wall while it is growing, weakening the wall and killing the bacteria. Below are the properties of penicillin:

- Chemical Formula: R-C₉H₁₁N₂O₄S
- Melting Point: 97 °C.
- Density: 1.41 g/ml
- Molar mass: 356.37g
- Percent Composition by mass: 57.4% C, 5.43% H, 8.38% N, 19.14% O, 9.59% S

![Figure 2.1: Molecular Structure of Penicillin](image-url)
2.2 Fed Batch Fermentation Process

Fed-batch fermentation is a production technique in between batch and continuous fermentation. A proper feed rate, with the right component constitution is required during the process. Fed-batch fermentation offers several advantages such as:

a) Able to produce high cell densities due to addition of working time (particularly important in the production of growth-associated products)
b) Controlled conditions in the provision of substrates during the fermentation, particularly regarding the concentration of specific substrates as for example the carbon source
c) Control over the production of by-products or catabolite repression effects due to limited provision of substrates solely required for product formation
d) The mode of operation can overcome and control deviations in the organism's growth pattern as found in batch fermentation
e) Allows the replacement of water loss by evaporation
f) Alternative mode of operation for fermentations leading with toxic substrates (cells can only metabolize a certain quantity at a time) or low solubility compounds
g) Increase of antibiotic-marked plasmid stability by providing the correspondent antibiotic during the time span of the fermentation
h) No additional special piece of equipment is required as compared with the batch fermentation mode of operation

A complete model of the penicillin fermentation must include the following aspects of the process:

- Smooth transition between growth and production phases
- Substrate limitation of growth
- Induction of penicillin production in response to stress
- Degradation of penicillin
- Oxygen limitation effects on growth and penicillin production
- Mass transfer limitations to substrate and oxygen transfer
2.3 Unstructured Model of Fed-Batch Fermentation

Fermentation processes can be modeled either by ‘structured’ models or by ‘unstructured’ models. Structured models represent the individual organisms in detail, but are usually mathematically too complex to be useful for controller design. Simple unstructured models can be obtained by assuming that the fermenter culture consists of a single, homogeneously growing organism. These models are well suited to the design of the controllers, since the models are given by a few nonlinear ordinary differential equations. A variety of fermentations can be described by the unstructured model. In this research, we concentrate on product optimization of a Penicillin fermentation process. The mathematical model of the fed-batch Penicillin fermentation will be discussed in chapter 2.4.

Figure 2.2: Fed-batch penicillin fermentation process (Birol et al., 2002)
2.4 Development Mathematical Model for Fed-batch Penicillin Fermentation

In this work, the unstructured model of fed-batch penicillin fermentation from Birol et al. (2002) works was chosen. This is because the form of these unstructured models fits well with any approach. They can be systematically identified and updated using data from experimental and production runs. In their work, the mechanistic model of Bajpai and Reuss (1980) was utilized as the starting point for model development. Additional input variables such as agitation power and aeration rate were included to extend the original model. The model is presented with better clarity in the next section.

2.4.1 Overall Mass Balance

The rate of change of mass in a fermenter can generally be represented by Equation (2.1).

\[
\begin{bmatrix}
\text{Rate of mass accumulated in the system}
\end{bmatrix} = \begin{bmatrix}
\text{Rate of mass by flow in the system}
\end{bmatrix} - \begin{bmatrix}
\text{Rate of mass by flow in the system}
\end{bmatrix} - \begin{bmatrix}
\text{Rate of mass consumed in the system}
\end{bmatrix} + \begin{bmatrix}
\text{Rate of mass generated in the system}
\end{bmatrix}
\]

(2.1)

Letting \( F \) denotes the volumetric flow rate of the entering feed stream and \( F_{out} \) denotes the volumetric flow rate of the exiting product stream, the overall material balance takes the following form:

\[
\frac{d(\rho V)}{dt} = \rho_{in} F - \rho_{out} F_{out}
\]

(2.2)
However, since this is a fed-batch fermentation, there is no outlet flow until the batch cycle is completed thus $F_{out} = 0$. Thus the total material balance takes the following form:

$$ \frac{d(\rho V)}{dt} = \rho_{in} F $$

(2.3)

In order to consider the effect of evaporative loss during fermentation, the term, $F_{loss}$ is included. The loss in volume due to evaporation is significant in industrial fermentations because the air entering the fermenter is fairly dry and it is about 90-100% relative humidity after bubbling through the broth. Typically, 10-20% of the total broth can be lost due to evaporation in one week fermentation process, the actual amount depending on the temperature of the fermentation (Birol et al., 2002). The effect of temperature and culture volume $V$ on the evaporative loss can be represented by the following equation:

$$ F_{loss} = V \lambda \left( e^{5(T_v - T_o)/T_v - T_o} - 1 \right) $$

(2.4)

Here $T_o$ and $T_v$ are the freezing and boiling temperatures of the culture medium respectively and are typically assumed to have same properties as water. Assuming that the evaporation rate can go to infinity at the boiling point, for engineering purposes the exponent 5 is considered large enough to represent this. Birol et al. (2002) suggested $\lambda$ is arranged to give an evaporation rate of $2.5 \times 10^{-4}$ l/h at the operation temperature (25 ºC).

In addition, the effect of acid/base addition on the total volume change of the culture broth, $F_{a/b}$ should also be included in Equation (2.3) as provided by Birol et al. (2002). The pH was kept constant at a value of 5.1 by adding highly concentrated (3 M) acid or base solution when necessary. By applying all these terms in Equation (2.3), the overall mass balance for a fermenter can be expressed as:

$$ \frac{d\rho V}{dt} = \rho_{in} F + \rho_{a/b} F_{a/b} - \rho_{loss} F_{loss} $$

(2.5)
Assuming that densities of entering liquid stream, the culture fluid, acid/base addition and evaporation rate in liquid form are both equal to $\rho$, the overall mass balance for a fermenter can be reduced to:

$$\frac{dV}{dt} = F + F_{a/b} - F_{\text{loss}} \tag{2.6}$$

### 2.4.2 Mass Balance on Biomass

A similar mass balance approach based on Equation (2.1) can be performed for the biomass in a fermenter. In fed-batch operation, $F_{\text{out}} = 0$; mass of biomass is the product of biomass concentration, $X$ multiplied by culture volume, $V$. Meanwhile the mass generated for certain time period is equal to $\mu XV$ where $\mu$ is the specific growth rate of biomass, and the rate of biomass death is equal to $k_d XV$ where $k_d$ is the specific death constant as explained by Bailey and Ollis (1986). Applying these terms in Equation (2.1) gives:

$$\frac{d(XV)}{dt} = FX_{\text{in}} + \mu XV - k_d XV \tag{2.7}$$

After expanding the differential and rearranging some terms in Equation (2.7), we obtain the following equation:

$$X \frac{dV}{dt} + V \frac{dX}{dt} = FX_{\text{in}} + (\mu - k_d) XV \tag{2.8}$$

Rearranging Equation (2.8) gives:

$$\frac{dX}{dt} = \frac{F}{V} X_{\text{in}} + \left(\frac{\mu - k_d}{V} - \frac{1}{V} \frac{dV}{dt}\right) X \tag{2.9}$$
Biomass in inlet flow, $X_{in}$, is equal to zero since the feed material is usually sterile. Meanwhile, the rate of biomass death is assumed to be negligible compared to growth so that $k_d \ll \mu$. Then Equation (2.9) becomes:

$$\frac{dX}{dt} = \mu X - \frac{X}{V} \frac{dV}{dt}$$

(2.10)

The specific growth rate $\mu$ can be described by Monod model as presented below, where the microorganisms’ growth rate depends on the concentration of limiting nutrient (Bailey and Ollis, 1986).

$$\mu = \mu_X \frac{S}{(K_X + S)}$$

(2.11)

$\mu_X$ represents the maximum specific growth rate, and $K_X$ is the substrate saturation constant. However, the Monod model is often not possible to describe the growth. It is only valid for balanced growth and should not be applied when growth conditions are changing rapidly. Therefore numerous modifications were made to Equation (2.11) to reduce the deviations of Monod model and measurements. One of the deviation forms of Equation (2.11) is Contois kinetics shown as Equation (2.12), which is used to represent the diffusion limitations that occur at high biomass concentrations.

$$\mu = \mu_X \frac{S}{(K_X X + S)}$$

(2.12)

In the Bajpai and Reuss model (1980), dissolved oxygen concentration $C_L$ and oxygen limitation constant $K_{OX}$ are included in Equation (2.12).

$$\mu = \mu_X \frac{S}{(K_X X + S)} \frac{C_L}{(K_{OX} X + C_L)}$$

(2.13)
According to Birol et al. (2002), effects of environmental variables such as pH and temperature should also be taken into account in the specific growth expression. These variables play an important role on the quality and quantity of the final product. By taking these variables into consideration, the specific growth rate can be expressed as:

\[
\mu = \frac{\mu_X}{1 + \left[ K_1 / [H^+] \right] + \left[ [H^+] / K_2 \right]} \left( \frac{S}{K_d X + S} \right) \left( \frac{C_L}{K_{dh} X + C_L} \right) \\
\left\{ k_e \exp \left( - \frac{E_e}{RT} \right) \right\} - \left\{ k_d \exp \left( - \frac{E_d}{RT} \right) \right\}
\]

(2.14)

2.4.2.1 Effect of Ph

The additional term in the specific growth rate expression Equation (2.14) is a typical inhibition term, which includes hydrogen ion concentration \([H^+]\) (Birol et al., 2002).

\[
\mu = f \left[ \frac{\mu_X}{1 + \left[ K_1 / [H^+] \right] + \left[ [H^+] / K_2 \right]} \right]
\]

(2.15)

Here, the values of \(K_1\) and \(K_2\) are chosen to be in the range of their typical values in the literature (Nielsen and Villadsen, 1994). Since the pH of the culture medium tends to become acidic, as the concentration of biomass increases; the amount of \(NH_4OH\) added into the culture medium also increases, in order to keep the pH constant during the penicillin fermentation. Based on this observation, the hydrogen ion concentration is related to biomass formation as:

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

(2.16)

Where \(B\) is given as:
Here, $F_a$ and $F_b$ represent acid and base flow rates in l/h, respectively, where the concentration in both solutions, $C_{a/b}$, are equal to be 3 M (Birol et al., 2002). Besides that, Birol et al. (2002) also suggested that under pH control, the hydrogen ion concentration can be calculated by taking the disassociation of water and acid/base into account as well as the hydrogen production. The proportionality constant, $\gamma$ is estimated as $10^{-5}$ mol [H$^+$]/g biomass.

### 2.4.2.2 Effect of Temperature

Temperature causes positive changes on the specific growth rate of a microorganism. An increase in temperature up to a certain value might cause a rapid decrease in biomass concentration. Here, the effect of temperature on the specific growth rate is given by Birol et al. (2002) as an Arrhenius type of kinetics:

\[
\mu = f \left\{ \left[ k_g \exp\left(\frac{-E_g}{RT}\right)\right] - \left[ k_d \exp\left(\frac{-E_d}{RT}\right)\right] \right\} 
\]

(2.18)

Here, $k_g$ 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. The gas constant, $R$ is 1.987 cal/(mol.K).
2.4.3 Mass Balance on Penicillin

For fed-batch operation, $F_{out}$ is equal to zero; penicillin concentration, $P_{in} = 0$ as there is no penicillin in the inlet flow to the fermenter. The mass of penicillin is equal to $PV$ where $P$ is the penicillin concentration and $V$ is the culture volume, the mass generation term in Equation (2.1) can be described as $\mu_{pp} XV$ where the $\mu_{pp}$ is the specific penicillin production rate, and the $KVP$ is the hydrolysis rate of penicillin where the $K$ is the penicillin hydrolysis constant. Substituting these terms into Equation (2.1) gives:

$$\frac{d(PV)}{dt} = \mu_{pp} XV - KPV$$

(2.19)

Expanding the differential and rearranging Equation (2.19) gives:

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

(2.20)

The specific penicillin production rate, $\mu_{pp}$ can be defined as (Birol et al., 2002):

$$\mu_{pp} = \mu_P \frac{S}{(K_P + S + S^2 / K_I)} \frac{C_L^P}{(K_{op}X + C_L^P)}$$

(2.21)

Here $\mu_P$ is the maximum specific penicillin production rate, $K_P$ is the inhibition constant, $K_I$ is the inhibition constant for product formation, $K_{op}$ is the oxygen limitation constant, and $C_L^P$ is the dissolved oxygen concentration.
2.4.4 Mass Balance on Substrate

Cells (biomass) consume substrate from external environment for growth and product synthesis requirements. The mass balance for the substrate can be represented by the following equation:

\[
\frac{d(SV)}{dt} = F_{s_f} - q_s X V \tag{2.22}
\]

Here, \(SV\) is the mass of substrate in the fermenter where \(S\) is the substrate concentration and \(V\) is the culture volume, \(F\) is the feed flow rate of substrate, \(s_f\) is the feed substrate concentration, \(q_s\) is the specific rate of substrate uptake, and \(X\) is the biomass concentration. By expanding the differential terms and some arrangements, Equation (2.22) can be rewritten as:

\[
\frac{dS}{dt} = -q_s X + \frac{F}{V} s_f - S \frac{dV}{dt} \tag{2.23}
\]

Patterns of substrate flow in cells synthesizing products depend on whether the product formation is directly linked to energy metabolism. When products are formed in energy-generating pathways such as in anaerobic culture, equation for rate of substrate consumption does not include a separate term for production; substrate requirements for product formation are already taken into account in terms of growth and maintenance-associated substrate uptake. In culture where product synthesis is only indirectly coupled to energy metabolism, rate of substrate consumption is a function of three factors: growth rate, product formation rate and substrate uptake rate for maintenance (Bailey and Ollis, 1986).

According to Bailey and Ollis (1986), a complete account of substrate uptake should include a maintenance component. Examples of maintenance functions are cell mobility, turnover of cellular components, and adjustment of membrane potential and internal pH. The specific rate of substrate uptake for maintenance activities is known as the maintenance coefficient, \(m_X\). Incorporating this term into
the computation of the specific rate of substrate uptake \( q_s \) yields the following relationships:

\[
- q_s = - \frac{\mu}{Y_{X/S}} - \frac{\mu_{pp}}{Y_{P/S}} - m_X
\]  

(2.24)

Substituting Equation (2.24) into Equation (2.23) yields:

\[
\frac{dS}{dt} = - \frac{\mu}{Y_{X/S}} X - \frac{\mu_{pp}}{Y_{P/S}} X - m_X X + \frac{F_{S_f}}{V} - \frac{S}{V} \frac{dV}{dt}
\]  

(2.25)

\( Y_{X/S} \) is the yield coefficient (g biomass/g substrate), \( Y_{P/S} \) is the yield coefficient (g penicillin/g substrate), \( \mu \) is the specific growth rate of biomass, \( \mu_{pp} \) is the specific production rate of penicillin, and \( m_X \) is the maintenance coefficient.

### 2.4.5 Mass Balance on Dissolved Oxygen

In aerobic culture, cells take up oxygen from the liquid. The rate at which cells in a fermenter consume oxygen determines the rate at which it must be transferred from gas (bubbles) to liquid (culture broth). Among the most important factors that influence the oxygen demand are cell species, culture growth phase, and nature of the carbon source in the medium. In batch culture, rate of oxygen uptake per volume of broth, \( Q_o \), varies with time. The reasons are: first, the concentration of cells increases during the course of batch culture and the total rate of oxygen uptake is proportional to the number of cells present. Secondly, the rate of oxygen consumption per cell, which is known as specific oxygen uptake rate, \( q_o \) also varies. The relationships between \( Q_o \) and \( q_o \) can be expressed as:

\[
Q_o = q_o X
\]  

(2.26)
The rate of oxygen transfer from the bubble to the cell is dominated by the rate of oxygen diffusing through the relatively stagnant liquid film surrounding the bubbles. The liquid film around the bubbles is regarded as a major resistance to oxygen transfer. In other words, the liquid-phase mass-transfer resistance dominates for cases involving solute that is poorly soluble in the liquid such as in the case of oxygen. The rate of change in dissolved-oxygen concentration $C_L$ during fermentation is equal to the rate of oxygen transfer from gas to liquid, minus the rate of oxygen uptake by the cells, $q_oXV$ as shown in Equation (2.27).

$$\frac{d(C_LV)}{dt} = k_LA(C^*_L - C_L) - q_oXV$$

(2.27)

Here, $C_LV$ is the mass of oxygen in fermenter where $C_L$ is the concentration of dissolved-oxygen and $V$ is the culture volume, $k_L$ is a transfer coefficient, and $A$ is the transfer area over which transport occurs. The difference $(C^*_L - C_L)$ between the maximum possible and actual oxygen concentrations in the liquid culture represents the concentration-difference driving force for mass transfer. Equation (2.27) can then be simplified to:

$$\frac{dC_L}{dt} = k_LA \left( C^*_L - C_L \right) - q_oX \frac{C_L}{V} \frac{dV}{dt}$$

(2.28)

The $(kLA/V)$ term can be represented by overall mass transfer coefficient $K_{la}$. By taking account for the effect of specific growth rate $\mu$, specific production rate of penicillin $\mu_{PP}$, and maintenance factor $m_o$ in specific oxygen uptake rate $q_o$, Equation (2.28) can be written in the form:

$$\frac{dC_L}{dt} = K_{la} \left( C^*_L - C_L \right) - \left( \frac{\mu}{Y_{X/O}} + \frac{\mu_{PP}}{Y_{P/O}} + m_o \right) \frac{C_L}{V} \frac{dV}{dt}$$

(2.29)

Equation (2.29) is then rearranged to give:
\[
\frac{dC_L}{dt} = -\frac{\mu}{Y_{X/O}} X - \frac{\mu_{pp}}{Y_{P/O}} X - m_X X + K_{la} \left(C_L^* - C_L\right) - \frac{C_L}{V} \frac{dV}{dt}
\]  
(2.30)  

Where \(Y_{X/O}\) is the yield constant with unit (g biomass/g oxygen), and \(Y_{P/O}\) is the yield constant with unit (g penicillin/g oxygen). The overall mass transfer coefficient \(K_{la}\) is constant in the original model of Bajpai and Reuss. However, in this work, \(K_{la}\) is assumed to be a function of agitation power input \(P_w\) and flow rate of oxygen \(f_g\) as suggested by Birol et al. (2002). This is represented by Equation (2.31). Here, the values of \(\alpha\) and \(\beta\) are constant for \(K_{la}\) so that the dependence of penicillin concentration on \(K_{la}\) showed a very similar behavior to the predictions of Bajpai and Reuss (Birol et al., 2002).

\[
K_{la} = \alpha \sqrt{f_g \left(\frac{P_w}{V}\right)^{\beta}}
\]  
(2.31)  

2.4.6 Mass Balance on Carbon Dioxide (CO₂)

CO₂ evolution is assumed to be due to growth, penicillin biosynthesis and maintenance requirements. This can be expressed as:

\[
\frac{dC_{CO₂}}{dt} = \alpha_1 \frac{dX}{dt} + \alpha_2 X + \alpha_3
\]  
(2.32)  

Here, \(C_{CO₂}\) is carbon dioxide concentration, \(\alpha_1\) is the constant relating CO₂ to growth, \(\alpha_2\) is the constant relating CO₂ to maintenance energy, and \(\alpha_3\) is the constant relating CO₂ to penicillin production. The values of \(\alpha_1\), \(\alpha_2\) and \(\alpha_3\) are chosen to give CO₂ profiles similar to the prediction of Montague et al. work (Birol et al., 2002).
2.4.7 Energy Balance

Based on conservation of energy principles, the overall energy balance equation can be written as:

\[
\begin{bmatrix}
\text{Rate of energy accumulated}
\end{bmatrix} = \begin{bmatrix}
\text{Rate of energy in by flow}
\end{bmatrix} - \begin{bmatrix}
\text{Rate of energy out by flow}
\end{bmatrix} - \begin{bmatrix}
\text{Rate of energy consumed in the system}
\end{bmatrix} + \begin{bmatrix}
\text{Rate of energy generated in the system}
\end{bmatrix}
\]

(2.33)

For a fermenter during normal operation, Equation (2.33) can be simplified as Equation (2.34):

\[
Q_{\text{acc}} = Q_{\text{rxn}} + Q_{\text{ag}} + Q_{\text{gas}} - Q_{\text{exch}} - Q_{\text{sen}}
\]

(2.34)

Here \(Q_{\text{acc}}\) is the heat accumulation rate by the system, \(Q_{\text{rxn}}\) is the heat generation due to microbial metabolism, \(Q_{\text{ag}}\) is the heat generation due to mechanical agitation, \(Q_{\text{gas}}\) is the heat generation due to aeration power input, \(Q_{\text{exch}}\) is the heat generation due to the surroundings and/or heat exchanger and \(Q_{\text{sen}}\) is the rate of sensible enthalpy gain by the flow system streams (exit – inlet). In typical fermentation process, changes in heats of mixing of substrate and products with the broth are generally negligible since cell-culture media are usually dilute aqueous solutions with behavior close to ideal. The effect of heat generation due to mechanical agitation \(Q_{\text{ag}}\) and aeration power input \(Q_{\text{gas}}\) are assumed to be negligible compared to the heat generation caused by microbial metabolism \(Q_{\text{rxn}}\) and heat exchanger \(Q_{\text{exch}}\). Therefore, Equation (2.34) can be written as:

\[
Q_{\text{acc}} = Q_{\text{rxn}} - Q_{\text{exch}} - Q_{\text{sen}}
\]

(2.35)
2.4.7.1 Rate of Accumulation

The accumulation term \( Q_{acc} \) can be written as:

\[
Q_{acc} = \frac{dE}{dt} = \frac{d}{dt} \left[ \rho V c_p (\Delta T) \right]
\]  

\( (2.36) \)

\( E \) represents the energy accumulated in the system. \( \rho \) is the density of culture volume, \( V \) is the culture volume, \( c_p \) is the heat capacity and \( \Delta T \) is the temperature difference between the temperature in the system and the reference temperature, \( (T - T_{ref}) \). By assuming \( \rho, c_p \) and \( T_{ref} \) are constant with respect to time, Equation (2.36) can now be expanded and then simplified to give:

\[
Q_{acc} = \frac{dE}{dt} = \rho c_p \left[ V \frac{dT}{dt} + (T - T_{ref}) \frac{dV}{dt} \right]
\]  

\( (2.37) \)

2.4.7.2 Sensible Heat

For fed-batch operation \( Q_{sen} \) can be represented by Equation (2.38) where \( M_i \) is the mass flows into the system, \( c_p \) is the heat capacity, \( T_f \) is the feed temperature of the substrate, and \( T_{ref} \) is the reference temperature.

\[
Q_{sen} = -M_i c_p (T_f - T_{ref})
\]  

\( (2.38) \)

Substituting Equation (2.37) and Equation (2.38) into Equation (2.35) yields:

\[
\rho c_p \left[ V \frac{dT}{dt} + (T - T_{ref}) \frac{dV}{dt} \right] = Q_{exch} - Q_{exch} - \left[ -M_i c_p (T_f - T_{ref}) \right]
\]  

\( (2.39) \)

\( M_i \) can be defined as \( \rho F \) where \( \rho \) is the density of the inlet (mass) flow and \( F \) is feed flow rate of the substrate. Here, the density \( \rho \) is assumed to be a constant. Equation (2.39) is then rearranged to give:
\[
\frac{dT}{dt} = \frac{F}{V} \left( T_f - T_{ref} \right) - \frac{1}{V} \left( T - T_{ref} \right) \frac{dV}{dt} + \frac{1}{V \rho c_p} \left( Q_{rxn} - Q_{exch} \right)
\]

(2.40)

\((dV/dt)\) is assumed to be equal to \(F\) since the effect of \(F_{a/b}\) and \(F_{loss}\) on heat generation is assumed to be negligible. Then, Equation (2.40) can be simplified to:

\[
\frac{dT}{dt} = \frac{F}{V} \left( T_f - T \right) + \frac{1}{V \rho c_p} \left( Q_{rxn} - Q_{exch} \right)
\]

(2.41)

2.4.7.3 Heat Input/Loss from Heat Exchanger

The energy balance model of a coiled type heat exchanger, which is suitable for a laboratory scale fermenter is given as follows (Birol et al., 2002):

\[
Q_{exch} = -\frac{aF_{c}^{b+1}}{F_c + (aF_{c}^{b} / 2\rho c_{pc})}
\]

(2.42)

By substituting Equation (2.42) into Equation (2.41) yields:

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

(2.43)

\(T_f\) is the feed temperature of substrate, \(F\) is the feed flow rate of substrate, \(F_c\) is the flow rate of the cooling liquid, \(\rho\) is the density of the culture medium, \(\rho_c\) is the density of the cooling liquid, \(c_p\) and \(c_{pc}\) represent the heat capacity of the culture medium and the cooling liquid respectively, \(Q_{rxn}\) is the heat of reaction while \(a\) and \(b\) are constants. For this particular equation, the unit of \(F\) is \(g/\) (liter.hr).

2.4.7.4 Heat of Reaction
Reactions in bioprocesses occur as a result of enzyme activity and cell metabolism. During reaction, bonds between atoms are rearranged. This results in relatively large changes in internal energy and enthalpy. Heat of reaction $\Delta H_{\text{rxn}}$ is defined as the energy released or absorbed during reaction, and is equal to the difference in enthalpy of reactants and products:

$$\Delta H_{\text{rxn}} = \sum_{\text{products}} M_h - \sum_{\text{reactants}} M_h$$ \hspace{1cm} \text{(2.44)}

$M$ is mass, and $h$ is specific enthalpy. For heat generation caused by microbial reactions/metabolism, Birol \textit{et al.} (2002) has suggested the following equation:

$$\frac{dQ_{\text{rnx}}}{dt} = r_{q1} \frac{dX}{dt} V + r_{q2} XV$$ \hspace{1cm} \text{(2.45)}

$(dQ_{\text{rnx}}/dt)$ is the volumetric heat production rate, $r_{q1}$ is assumed to be constant and might be treated as a yield coefficient, and $r_{q2}$ is a constant for heat production during maintenance. The second term in Equation (2.45) is important to consider since metabolic maintenance activities give a significant effect on the heat generation. According to Birol \textit{et al.} (2002), the heat generation and CO$_2$ evolution show similar profiles. So, their production rate due to growth $(dX/dt)$ and biomass $(X)$ should have the same ratio as a first approximation. This observation has enabled the value of $r_{q2}$ to be calculated.

\textbf{2.4.8 Penicillin Fed-batch Process Model Equation}
By substituting all the components of the total overall and energy balance into Equation (2.1) and (2.33), mathematical models for the penicillin fed-batch process can be summarized as:

\[
\frac{dV}{dt} = F + F_{\text{a/b}} - F_{\text{loss}} \quad (2.46)
\]

\[
\frac{dX}{dt} = \mu X - \frac{X}{V} \frac{dV}{dt} \quad (2.47)
\]

\[
\frac{d[H^+]}{dt} = \gamma \left( \frac{\mu X - FX}{V} \right) + \left[ \frac{-B + \sqrt{B^2 + 4 \times 10^{14}}}{2} - [H^+] \right] \frac{1}{\Delta t} \quad (2.48)
\]

\[
\frac{dP}{dt} = \mu_{pp} X - K_P \frac{P}{V} \frac{dV}{dt} \quad (2.49)
\]

\[
\frac{dS}{dt} = -\frac{\mu}{Y_{X/S}} X - \frac{\mu_{pp}}{Y_{P/S}} X - m_X X + \frac{F_{S_f}}{V} - \frac{S}{V} \frac{dV}{dt} \quad (2.50)
\]

\[
\frac{dC_L}{dt} = -\frac{\mu}{Y_{X/L}} X - \frac{\mu_{pp}}{Y_{P/L}} X - m_X X + K_{la} (C_{L_0} - C_L) - \frac{C_L}{V} \frac{dV}{dt} \quad (2.51)
\]

\[
\frac{dC_{\text{coz}}}{dt} = \alpha_1 \frac{dX}{dt} + \alpha_2 X + \alpha_3 \quad (2.52)
\]

\[
\frac{dT}{dt} = \frac{F}{s_f} \left( T_f - T \right) + \frac{1}{V \rho c_p} \left[ Q_{\text{rem}} - \frac{aF_{c}^{b+l}}{F_c} \left( aF_c^b / 2 \rho_c c_p \right) \right] \quad (2.53)
\]

\[
\frac{dQ_{\text{rem}}}{dt} = r_{q_1} \frac{dX}{dt} V + r_{q_2} XV \quad (2.54)
\]

\[
F_{\text{loss}} = V \lambda \left( e^{s(T-\text{To})/(T_f-\text{To})} - 1 \right) \quad (2.55)
\]
\[ \mu = \left[ \frac{\mu_x}{1 + [K_t/H^+] + [H^+] K_2} \right] \frac{S}{(K_x X + S)(K_{oX} X + C_L)} \left( k_g \exp\left(-\frac{E_g}{RT}\right) - k_d \exp\left(-\frac{E_d}{RT}\right) \right) \]  

(2.56)

\[ B = \left[ \frac{10^{-14} / [H^+] - [H^+] V - C_{alb} (F_a + F_b) \Delta t}{V + (F_a + F_b) \Delta t} \right] \]  

(2.57)

\[ \mu_{pp} = \mu_p \frac{S}{S + S^2 / K_t} \frac{C_L^p}{(K_{oX} X + C_L^p)} \]  

(2.58)

\[ K_{la} = \alpha \sqrt{f_d \left( \frac{P_n}{V} \right)} \]  

(2.59)

2.5 Model Predictive Control