Mathematical Modelling and Analysis of Dynamic Behaviour of a Fed-batch Penicillin G Fermentation Process

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Abstract

This paper discusses the characteristics of penicillin G fermentations with substrate inhibition using dynamic simulations. The fermentation process was simulated in MATLAB environment and analyses of the dynamic behaviour of the process were carried out to provide the necessary process insights. Dynamic simulations revealed variations in the sensitivity of the model to substrate concentrations, depending on the operating point. This variation indicates non-linearity in dynamic behaviour. PID control was also evaluated and results are demonstrated.

Keywords:

Penicillin G; Fermentation; Dynamic simulation; PID controller

Introduction

Penicillin is an antibiotic that is categorised as a secondary metabolite product that has played important roles in pharmaceutical field for years. Generally, the industrial production of penicillin is characterised by two distinct regimes. The initial phase is to grow cells in a batch culture. This is then followed by a fed-batch operation to promote the biosynthesis of penicillin. In fermentation, substrate is very important for cell growth, maintenance of the microbial population and formation of products. However, for some processes, product formations may be suppressed when substrate concentration exceeds certain value. As a result, controlled supply of substrate is necessary.

In order to explore the effects of process variables and analyse the sensitivity of these variables, the use of dynamic simulation techniques is advantageous. This has motivated some active researchers. For example, mechanistic models of penicillin fermentation had been developed by Bajpai and Reuβ [1]. In addition, there were also researchers working on developing software package [2], hybrid modelling ([3],[4],[5]) and dynamic optimization [6].

In this work, we concentrate on the development of a complete mathematical model and simulation test-bed for process control studies on a Penicillin G fermentation process. The mechanistic model of Bajpai and Reuβ [1], and the extended model by Birol et al.[2] were utilized as the basis of our modelling efforts. These models are classified as unstructured model and they are simpler than structured models. In unstructured models, all cellular physiology information is gathered in a single biomass term so that there is no explicit structural information about the cellular activity [2]. On the other hand, structured models for penicillin production include the effects of cell physiology on penicillin production.

Mathematical Modelling

Mathematical Modelling of Penicillin Fermentation

In a fed-batch operation, there is no removal of products from the fermenter until the end of the process. The output of a fermenter during penicillin production, F_{out}, is therefore equal to zero (see Equation (1)). The nutrients are fed at a variable rate to the culture broth in a fed-batch process [7].

$$F_{out} = 0 \tag{1}$$

The reactions involved are simplified as follow:

$$S \rightarrow X; S \rightarrow P; \tag{2}$$

In this case, the manipulated variable is the feed rate of S, which is the substrate.

Overall Mass Balance

The overall mass balance for the fermentation process was simplified and shown in Equation (3) where V represents the culture volume, and F is the feed rate of substrate.

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

The equation above indicates that a constant density assumption is made. Because penicillin production is an

aerobic fermentation process, the fermentation culture is continuously sparged with air. The air leaves the fermenter through the exhaust gas line. If the air entering the fermenter is dry, water is continually stripped from the medium and leaves the system as vapour. Evaporative water loss can be significant over a period of days [8]. Typically, 10-20% of the total broth can be lost due to evaporation during one week of fermentation, the actual amount depending on the temperature of the fermentation [2]. Hence, evaporative loss, F_{loss} should be taken into account in modelling of a fermenter. 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 (3). By including all these terms, the overall mass balance for a fermenter can be expressed as:

$$\frac{dV}{dt} = F + F_{a/b} - F_{loss} \tag{4}$$

The suggested evaporative loss can be represented by the following relationships [2]:

$$F_{loss} = V.\lambda(e^{5((T-To)/Tv-To)}-1) \tag{5}$$

Here, T_o and T_v are the freezing and boiling temperature of the culture medium that were assumed to have the same properties as water, respectively, and λ is a suggested evaporation rate of 2.5 x 10^{-4} l/h at the operation temperature of 25 °C [2]. Here, the rate of evaporation approaches infinity at the boiling point, and for engineering purposes the exponent 5 is large enough to represent this [2].

Mass Balance on Biomass

The equation for mass balance on biomass is simplified as:

$$\frac{dX}{dt} = \mu X - \frac{X}{V}\frac{dV}{dt} \tag{6}$$

where X is the biomass concentration and μ is the specific growth rate of the biomass. The specific growth rate can be described by Monod model (Equation (7)), where the microorganisms' growth rate depends on the concentration of limiting nutrient ([8];[9];[10]; [11]).

$$\mu = \mu_x \frac{S}{(K_x + S)} \tag{7}$$

Here, μ_X represents the maximum specific growth rate, and K_X is the substrate saturation constant. However, the Monod model is inaccurate to describe the growth under certain conditions [12]. The model is only valid for balanced growth and should not be applied when growth conditions are changing rapidly [8]. Numerous modifications were made to Monod model to improve accuracy of the kinetic model. One example is the Contois kinetics that is used to represent the diffusional limitations that occur at high biomass concentrations ([10];[12]). The Contois kinetics model is given by equation (8) below.

$$\mu = \mu_x \frac{S}{(K_x X + S)} \tag{8}$$

For the Bajpai and Reuβ model [1], dissolved oxygen concentration C_L and oxygen limitation constant K_{OX} are included in Equation (8) to give:

$$\mu = \mu_x \frac{S}{(K_x X + S)}\frac{C_L}{(K_{OX} X + C_L)} \tag{9}$$

According to Birol *et al.* [2], effects of environmental variables such as pH and temperature should also be taken into account in the specific growth expression. These variables play important roles on the quality and quantity of the final product. Considering these variables, the specific growth rate is now given by:

$$\mu = \left[\frac{\mu_x}{1+[K_1/[H^+]]+[[H^+]/K_2]}\right]\frac{S}{(K_x X + S)}\frac{C_L}{(K_{OX} X + C_L)} \left\{\left[k_g \exp\left(-\frac{E_g}{RT}\right)\right]-\left[k_d \exp\left(-\frac{E_d}{RT}\right)\right]\right\} \tag{10}$$

Effect of pH

Another term to consider in the specific growth rate expression is the hydrogen ion concentration $[H^+]$ [2]. The pH of the culture medium becomes 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 [2]:

$$\frac{d[H^+]}{dt} = \gamma\left(\mu X - \frac{FX}{V}\right)+\left[\frac{-B+\sqrt{(B^2+4\times10^{-14})}}{2}-[H^+]\right]\frac{1}{\Delta t} \tag{11}$$

B is given as:

$$B = \frac{[10^{-14}/[H^+]-[H^+]]V - C_{a/b}(F_a + F_b)\Delta t}{V + (F_a + F_b)\Delta t} \tag{12}$$

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 assumed equal to be 3 M. Besides that, Birol *et al.* [2] 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, γ is estimated as 10^{-5} mol $[H^+]$/g biomass.

Effect of Temperature

According to Doran [8], temperature has a significant kinetic effect on reactions. The effect of temperature on the specific growth rate can be represented by an Arrhenius type of kinetics:

$$\mu \propto f\left\{\left[k_g \exp\left(-\frac{E_g}{RT}\right)\right]-\left[k_d \exp\left(-\frac{E_d}{RT}\right)\right]\right\} \tag{13}$$

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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.

Temperature Control

The temperature of the culture medium was kept constant at 25 °C. It was controlled by a split-range PID controller that allows the heating or cooling water flow rate to be manipulated. The velocity form of the digital PID algorithm (as shown in Equation (14)) was used.

$$\Delta MV_N = K_C(E_N - E_{N-1} + \frac{\Delta t}{\tau_I}E_N - \frac{\tau_d}{\Delta t} \times(CV_N - 2CV_{N-1} + CV_{N-2}))MV_N = MV_{N-1} + \Delta MV_N \quad (14)$$

Here, K_C is the proportional gain, τ_I is the integral constant, τ_d is the derivative constant and CV_N, SP_N and MV_N represent the current values of the controlled variable, set point and controller output at the current sample N, respectively, with the current value of error, E_N $(= SP_N - CV_N)$.

Mass Balance on Penicillin

For a fed-batch fermentation process with penicillin concentration, P and culture volume, V, specific penicillin production rate, μ_{PP}, and penicillin hydrolysis constant, K, the mass balance equation on penicillin is reduced to:

$$\frac{dP}{dt} = \mu_{PP}X - KP - \frac{P}{V}\frac{dV}{dt} \quad (15)$$

The specific penicillin production rate, μ_{PP} is defined as Equation (16) [1].

$$\mu_{PP} = \mu_P \frac{S}{(K_P + S + S^2/K_I)} \frac{C_L^P}{(K_{OP}X + C_L^P)} \quad (16)$$

where μ_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.

Mass Balance on Substrate

A mass balance on substrate is shown as follow:

$$\frac{dS}{dt} = -\frac{\mu}{Y_{X/S}}X - \frac{\mu_{PP}}{Y_{P/S}}X - m_X X + \frac{Fs_f}{V} - \frac{S}{V}\frac{dV}{dt} \quad (17)$$

Here, S and X is the substrate and biomass concentration respectively, $Y_{X/S}$ is the yield coefficient (g biomass/g substrate), $Y_{P/S}$ is the yield coefficient (g penicillin/g substrate), μ is the specific growth rate of biomass, μ_{PP} is the specific production rate of penicillin, m_X is the maintenance coefficient, F is the feed flow rate of substrate, s_f is the feed substrate concentration, and V is the culture volume.

Mass Balance on Dissolved Oxygen

Mass balance on dissolved oxygen can be written as:

$$\frac{dC_L}{dt} = -\frac{\mu}{Y_{X/O}}X - \frac{\mu_{PP}}{Y_{P/O}}X - m_o X + K_{la}(C_L^* - C_L) - \frac{C_L}{V}\frac{dV}{dt} \quad (18)$$

where μ is the specific growth rate, μ_{PP} is the specific production rate of penicillin, C_L is the concentration of dissolved-oxygen and V is the culture volume, $Y_{X/O}$ is the yield constant with unit (g biomass/g oxygen), $Y_{P/O}$ is the yield constant with unit (g penicillin/g oxygen), K_{la} is the overall mass transfer coefficient. The difference $(C_L^* - C_L)$ between the maximum possible and actual oxygen concentrations in the liquid culture represents the concentration driving force for mass transfer.

The overall mass transfer coefficient K_{la} is constant in the original model of Bajpai and Reuβ [1]. 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 Bailey and Ollis [13]. This is shown in Equation (19). The value of α and β are assigned so that the dependence of penicillin concentration on K_{la} matches closely to the predictions of Bajpai and Reuβ [1].

$$K_{la} = \alpha\sqrt{f_g}\left(\frac{P_w}{V}\right)^{\beta} \quad (19)$$

Mass Balance on Carbon Dioxide (CO_2)

During a fermentation process, CO_2 is produced. The CO_2 evolution rate can be represented by :

$$\frac{dCO_2}{dt} = \alpha_1\frac{dX}{dt} + \alpha_2 X + \alpha_3 \quad (20)$$

Here, α_1 is the constant relating CO_2 to growth, α_2 is the constant relating CO_2 to maintenance energy, and α_3 is the constant relating CO_2 to penicillin production. The values of α_1, α_2 and α_3 are chosen to give CO_2 profiles similar to the predictions of Montague and co-workers[5], as suggested by Birol *et al.* [2].

Energy Balance

In fermentation, 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 behaviour close to ideal [8]. In addition, the effects of heat generation due to mechanical agitation and aeration power input are also assumed to be negligible compared to the heat generation caused by microbial metabolism, the surroundings, heat exchanger, and rate of sensible enthalpy gain by the flow system streams. The energy balance on a coiled type heat exchanger, which is suitable for a laboratory scale fermenter has been suggested as follow [2]:

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$$\frac{dT}{dt} = \frac{F}{s_f}(T_f - T) + \frac{1}{V\rho c_p}\left[Q_{rxn} - \frac{aF_c^{b+1}}{F_c + \left(aF_c^b / 2\rho_c c_{pc}\right)}\right] \quad (21)$$

Here, T_f is the feed temperature of the substrate, F is the feed flow rate of the substrate, F_c is the flow rate of the cooling liquid, ρ is the density of the culture medium, ρc 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, a and b are constants. For this particular equation, the unit of F is g/(l.hr).

Heat of Reaction

Reactions in bioprocesses occur as a result of enzyme activity and cell metabolism. For heat generation caused by microbial reactions/metabolism, Birol *et al.* [2] has suggested the following equation:

$$\frac{dQ_{rxn}}{dt} = r_{q1}\frac{dX}{dt}V + r_{q2}XV \quad (22)$$

(dQ_{rxn}/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 (22) is important to take account for the heat production during maintenance since metabolic maintenance activities give a significant effect on the heat generation.

Process Simulation

The model developed here was simulated using MATLAB software. The ordinary differential equations were solved using Fourth-Order Runge-Kutta algorithm with adaptive step size mechanism. Sampling time was fixed at 0.02 hour as suggested by Birol *et al.* [2]. The work of Birol *et al.* was regarded as the benchmark for the simulation study and as such, the kinetic parameters as well as the initial values were based on their work. These are tabulated in Tables 1 and 2.

Table 1 - Kinetic Parameters and Variables [2]

Time, t (h)		Value	Time, t (h)		Value
a	Heat transfer coefficient of heating/cooling liquid (cal/h.°C)	1000	r_{q2}	Constant in heat generation (cal/g biomass.h)	1.6783 x 10^{-4}
b	Constant	0.60	s_f	Feed substrate concentration (g/l)	600
E_d	Activation energy for cell death (cal/mol)	50 000	T_f	Feed temperature of substrate (K)	298
E_g	Activation energy for growth (cal/mol)	5100	$Y_{P/O}$	Yield constant (g penicillin/g oxygen)	0.20
k_d	Arrhenius constant for cell death	10^{33}	$Y_{P/S}$	Yield constant (g penicillin/g glucose)	0.90
k_g	Arrhenius constant for growth	7 x 10^3	$Y_{X/O}$	Yield constant (g biomass/g oxygen)	0.04
K	Penicillin hydrolysis rate constant (h^{-1})	0.04	$Y_{X/S}$	Yield constant (g biomass/g glucose)	0.45
K_1	Constant (mol/l)	10^{-10}	α	Constant in K_{la}	70
K_2	Constant (mol/l)	7 x 10^{-5}	α_1	Constant relating CO_2 to growth (mmol CO_2/g biomass)	0.143
K_I	Inhibition constant for product formation (g/l)	0.10	α_2	Constant relating CO_2 to maintenance energy (mmol CO_2/g biomass.h)	4 x 10^{-7}
K_{OP}	Oxygen limitation constant (with limitation)	5 x 10^{-5}	α_3	Constant relating CO_2 to penicillin production (mmol CO_2 /l.h)	10^{-4}
K_{OX}	Oxygen limitation constant (with limitation)	2 x 10^{-2}	β	Constant in K_{la}	0.4
K_P	Inhibition constant (g/h)	0.0002	γ	Proportionality constant (mol $[H^+]$/g biomass)	10^{-5}
K_X	Saturation constant (g/l)	0.15	μ_X	Maximum specific growth rate (h^{-1})	0.092
m_o	Maintenance coefficient on oxygen (h^{-1})	0.467	μ_P	Specific rate of penicillin production (h^{-1})	0.005
m_X	Maintenance coefficient on substrate (h^{-1})	0.014	λ	Constant in F_{loss} (h^{-1})	2.5x10^{-4}
p	Constant	3	ρc_p	Density of medium (g/l) x Heat capacity of medium (cal/g.°C)	1/1500
r_{q1}	Yield of heat generation (cal/g biomass)	60	ρ_c c_{pc}	Density of cooling liquid (g/l) x Heat capacity of cooling liquid (cal/g.°C)	1/2000

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Table 2 - Initial Conditions [2]

	Time, t (h)	Value
C_L	Dissolved oxygen concentration (= C_L^* at saturation) (g/l)	1.16
CO_2	Carbon dioxide concentration (mmol/l)	0.5
$[H^+]$	Hydrogen ion concentration (mol/l)	$10^{-5.1}$
P	Penicillin concentration (g/l)	0
Q_{rxn}	Heat generation	0
S	Substrate concentration (g/l)	15
T	Temperature (K)	297
V	Culture volume (l)	100
X	Biomass concentration (g/l)	0.1

The study was divided into two parts:

1. dynamic simulation of batch culture
2. closed-loop operation of fed-batch fermentation. Here, pH and temperature were controlled using algorithm.

Results and Discussion

The profiles of the output variables under nominal operating conditions for batch fermentation process are shown in Figure 1 to Figure 8. The production of penicillin started only after a lag-phase. According to Doran [8], cells use the lag phase to adapt to their new environment. Following the lag period, the growth entered the acceleration phase. After the substrate in the culture medium depleted, cell growth slowed down, showing a decreasing trend as shown in Figure 2. The penicillin concentration started to increase at this stage due the increase amount of penicillin (see Figure 3).This indicates that the process was in the product formation phase.

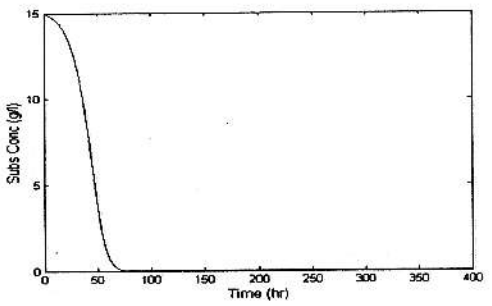

Figure 1 – Profile of substrate concentration

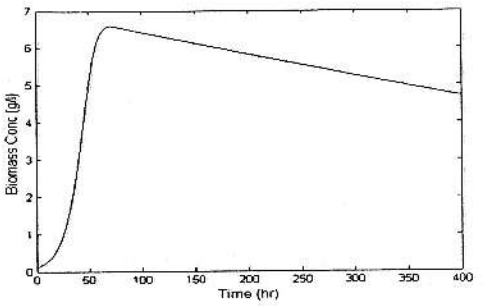

Figure 2 – Profile of biomass concentration

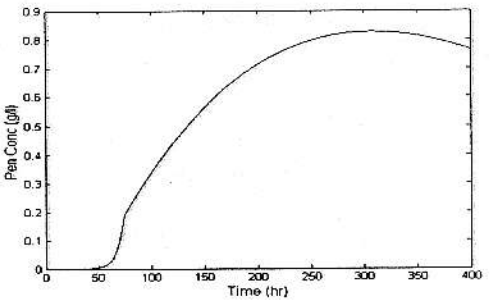

Figure 3 – Profile of penicillin concentration

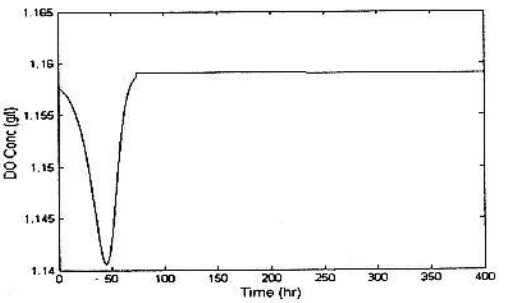

Figure 4 – Profile of dissolved oxygen concentration

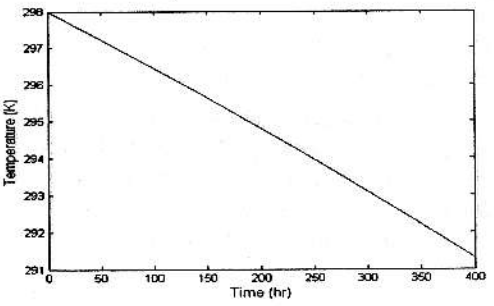

Figure 5 – Profile of temperature

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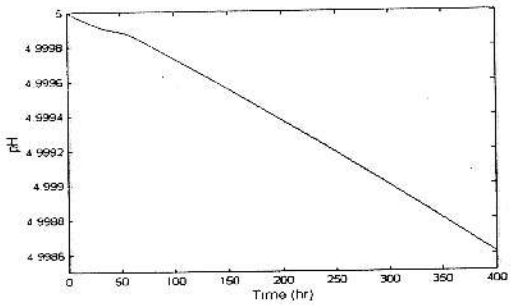

Figure 6 – Profile of pH

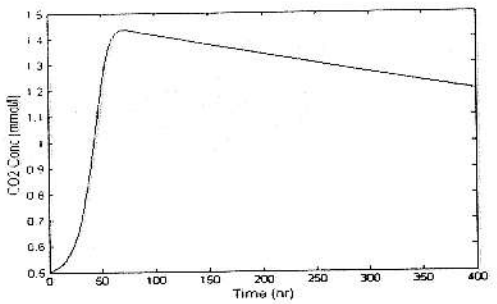

Figure 7 – Profile of carbon dioxide concentration

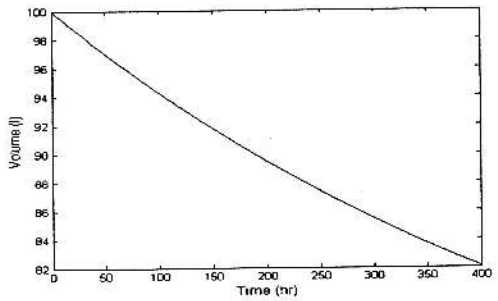

Figure 8 – Profile of culture volume

During the fermentation process, dissolved oxygen was utilised for cell respiration (see Figure 4) and carbon dioxide was released by the cells. The concentrations of gasses in the system also serve as good indicators on problem statement.

It can also be observed that temperature and pH were reduced with time (see Figure 5 and 6). This may directly affect the quality of the process. A control system is therefore required to maintain these values.

The simulation results for different values of initial substrate concentration are illustrated in Figure 9 and Figure 10. Here, it can be seen that the penicillin production increases with an increase in the initial substrate concentration, and then levels off due to substrate limitation in batch fermentation.

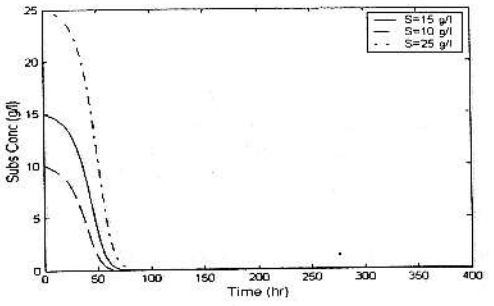

Figure 9 – Substrate concentration at initial substrate concentrations of 10, 15 and 25 g/l.

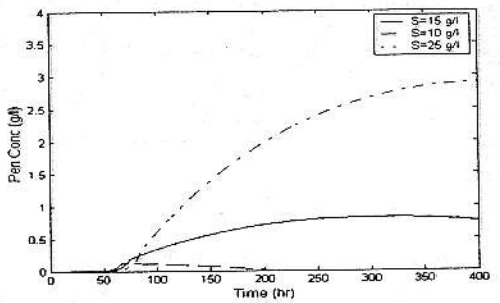

Figure 10 - Penicillin Concentration at initial substrate concentrations of 10, 15 and 25 g/l.

Fed-batch Fermentation

Fed-batch fermentation was accomplished by continuously feeding the substrate to promote the biosynthesis of the product. Here, a threshold value of 0.5 g/l was assigned to the substrate concentration. Substrate feeding was activated once the substrate concentration reached this threshold value. However, high concentration of substrate may inhibit the cell growth. Hence, controlled supply of carbon source (glucose) was practiced in this study.

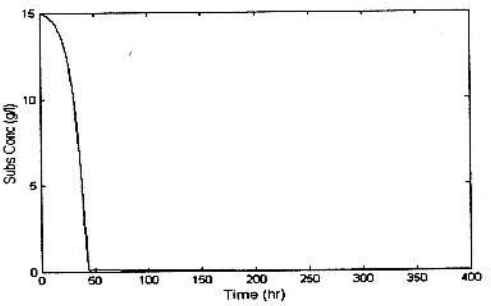

Figure 11 – Profile of substrate concentration

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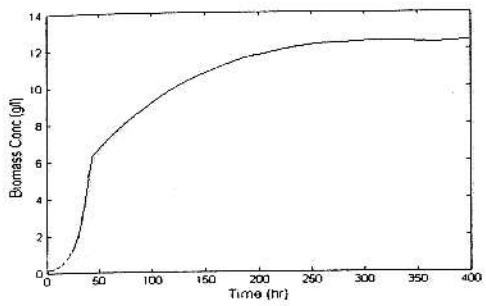

Figure 12 – Profile of biomass concentration

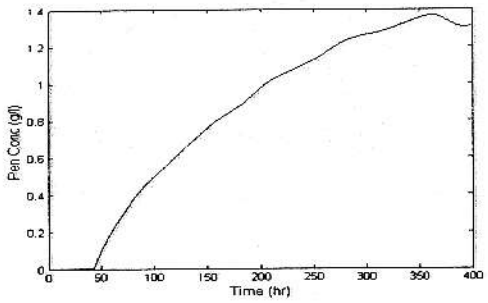

Figure 13 – Profile of penicillin concentration

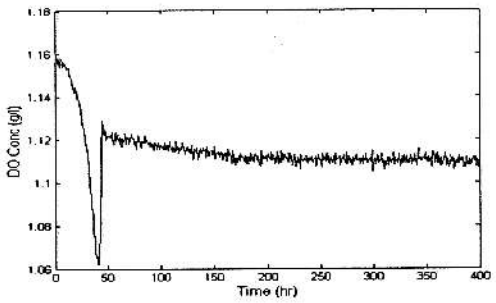

Figure 14 – Profile of dissolved oxygen concentration

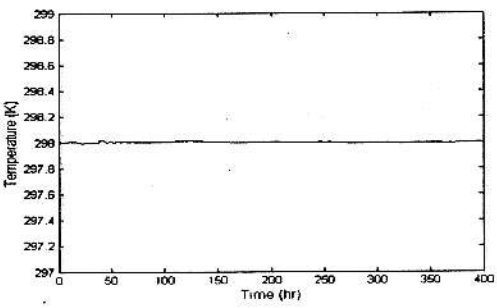

Figure 15 – Profile of temperature

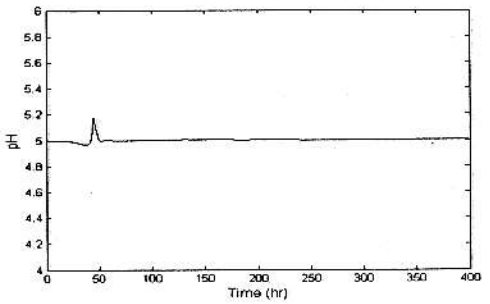

Figure 16 – Profile of pH

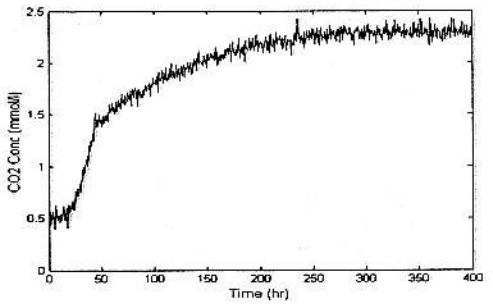

Figure 17 – Profile of carbon dioxide concentration

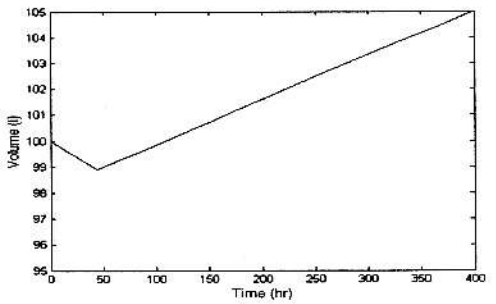

Figure 18 – Profile of volume

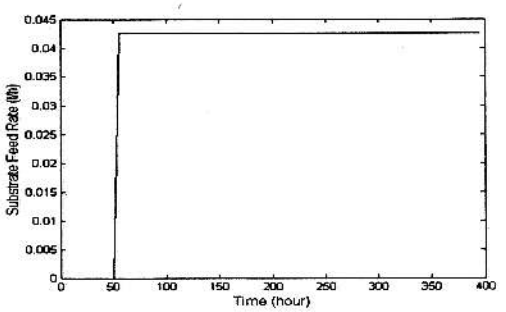

Figure 19 – Profile of substrate feed rate

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In this work, the substrate feed rate, pH, and temperature were controlled using PID controllers. The profiles of the output variables under nominal operating condition, where constant glucose feed are used during the fed-batch operation, are illustrated in Figure 11 to Figure 19. Note that both biomass and penicillin concentrations had been improved by adding a slow glucose feed into the batch fermenter. The controlled supply of glucose enabled both growth and penicillin production to take place simultaneously [1].

By including PID controllers in the system, the values of pH and temperature were maintained close to their desired set points. Compared to the results without controllers, the variations from the set-point values had been reduced. As a result, the overall performance of the fermentation process was improved. This proved the importance of control system for the process.

Conclusion

An unstructured model for a fed-batch Penicillin G fermentation process had been developed in this study. This mathematical model includes additional input variables like feed flow rate of substrate, pH, temperature, aeration rate, agitation power as well as output variables such as CO_2 evolution and heat generation terms. The model was simulated in MATLAB environment and analyses of the dynamic behaviour of the process were carried out. From the observation, a good agreement was shown between the results obtained in this study and the literature.

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