Optimal feeding strategy of Cephalosporin C fermentation

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Optimal feeding strategy of Cephalosporin C fermentation

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Abstract. Cephalosporin C is a β-lactam type of antibiotic produced from the fungus *Acremonium chrysogenum* through fermentation process, either by batch or fed-batch mode. Cephalosporin represents the bulk majority of antibiotic production due to its enhanced antibacterial spectrum against gram positive and gram negative bacterial strains relating to diseases and infections in the skin, respiratory system, and urinary tract. In the production process, it is crucial to maximize the yield of antibiotic produced, since the major costs of production come from the fermentation and recovery of the antibiotic. To address this issue, an effective feeding strategy of the culture producing the target antibiotic is very important to achieve high yield and avoid undesired production of other metabolites, which reduce the yield of desired metabolite. The latter can add extra cost to recovery and purification of the desired metabolite. This paper presents an optimal strategy for the fed-batch fermentation process producing Cephalosporin C (CPC) from the fungus *A. chrysogenum* by optimization of the substrate feeding flow rate. The preferred substrates are glucose and sucrose as the fungus possesses diauxic behaviour in the presence of the two carbon sources. It is shown that a two-step fed-batch feeding of glucose results in a significantly higher antibiotic production than a batch mode or single-step fed-batch feeding strategy.

1. Introduction

Acremonium chrysogenum also known as *Cephalosporin acremonium* (old name), is a fungus commonly found in nature – when colonies of white and grey interwoven hyphae are formed from overgrown soil, plant debris, and organic matter in humid environment [1]. Fermentation of this fungus will result in a production of CPC, which is used as antibiotics. Currently, cephalosporin is widely used to treat diseases caused by bacterial infections that are caused by gram positive or gram negative bacteria in the respiratory system, urinary tract, and skin [2]. It is one of the major products for antibiotics in the pharmaceutical industry, with a total world market of about $10 billion – this success is due to superior properties of cephalosporin over that of the penicillin in areas, such as resistance to beta lactamase, broad spectrum, and low toxicity [3].

Cephalosporin is produced through fermentation of *A. chrysogenum* under the presence of oxygen (aerobic conditions), carried out in a conventional method using free or immobilized fungi under solid-state or surface-liquid fermentation that is operated in a bioreactor [3]. The fermentation process is carried out in a chemically defined medium with the presence of carbon sources, usually glucose and/or sucrose. The *A. chrysogenum* possesses a diauxic behavior (or biphasic growth) of substrate consumption due to the subsequent usage of the carbon sources. Initially, the glucose is consumed first by the cells as a primary carbon source for as long as glucose is still present in the system until it is

completely consumed, thenceforth, sucrose becomes the secondary carbon source for the purpose of cell growth and maintenance [4]. The *A. chrysogenum* displays four morphological forms that produces cephalosporin, which are slim thin-walled filamentous hyphae (mycelia), swollen hyphal fragments, and arthrospores [5].

The production of cephalosporin starts when then glucose concentration depletes and the fragmentation of swollen hyphal fragments develops into arthrospores, via which the Cephalosporin is produced and the production phase is maintained. The production ends when all of the swollen hyphal fragments are fully developed and differentiated into arthropores and as the carbon sources completely depleted [4].

The goal of the present work is to determine an optimal feeding strategy for the cephalosporin fermentation. Since rigorous model-based optimization is not practical due to discontinuity in the process dynamics, a trial-and-error approach optimization will be conducted using step test strategy. So far, there has been very limited study conducted on the optimization of cephalosporin fermentation by the *A. chrysogenum.*

2. Methodology

This research consists of four steps of study; the first step is select a suitable mathematical model, second step is to conduct trial and error of feeding flowrate by step test, third step is to perform the principal component analysis (PCA), and the fourth step is determine the optimal feeding strategy.

2.1. Mathematical Model Selection and Model Validation

A mathematical model selection is done through literature review to the gather relevant information and analyze existing models relating to the study. It is then followed by model validation, to ensure if the mathematical model run in the simulation shows fitting results to the mathematical model chosen. This model will be used as a base case.

The mathematical model chosen for his work is based on the stoichiometric and rate equations proposed by Basak *et al*. [6]. This model is used to describe the kinetics of the fungus in the production of Cephalosporin C (CPC) from *A. chrysogenum*. The model represents the diauxic growth properties of the fungus in the presence of two carbon sources, glucose and sucrose. Moreover, a novel lag model is incorporated in order to describe the delay effects between the sugars and the CPC yield.

A system model is then established in order to perform fundamental mass-energy balance modelling of the fed-batch bioreactor. Hence, to understand the major dynamics behavior pertaining to the open-loop fed-batch bioreactor in which fungal cultivation takes place. In this model, the total cell mass (X) of *A. chrysogenum* is assumed to consist of two cell types, which are thin hyphae (X_H) and thick walled cells (X_T). The cells have irreversible morphological differentiation of $X_H \rightarrow X_T$.

The biomass accumulation is as follows

$$X = X_H + X_T \tag{1}$$

$$\frac{dX_H}{dt} = \mu_H X - \mu_T X_H - \delta_H X_H \tag{2}$$

$$\frac{dX_T}{dt} = \mu_T X_H - \delta_T X_T \tag{3}$$

The enzyme accumulation profile is given

$$\frac{dE}{dt} = \propto \mu_T X_H - \beta E \tag{4}$$

The consumption rate of glucose (S_1) and sucrose (S_2) are expressed in the forms of

$$\frac{dS_1}{dt} = \frac{-\mu_{S1} X}{Y_{S1}} \tag{5}$$

$$\frac{dS_2}{dt} = \frac{-\mu_{S2} X}{Y_{S2}} - \frac{m_{S2} S_2 X}{K_{S2} + S_2 + I_2 S_1} \tag{6}$$

Here, the specific growth rates for thick-walled cells μ_T and thin-walled cells μ_H given as follows

$$\mu_T = \frac{\mu {T}^m}{1+I_1 S_1} \tag{7}$$

$$\mu_H = \begin{Bmatrix} \mu_{S1} & t \le t_1 \\ \mu_{S2} & t > t_1 \end{Bmatrix} \tag{8}$$

The specific growth rates related to glucose μ_{S1} and sucrose μ_{S2} are written in terms of

$$\mu_{S1} = \frac{\mu {S_1}^m S_1}{K_{S1}+S_1} \tag{9}$$

$$\mu_{S2} = \phi_{lag} \left[\frac{\mu {S_2}^m S_2}{K_{S2}+S_2}\right] \tag{10}$$

The corresponding lag function (ϕ_{lag}) and lag period (t_{lag}) are

$$\phi_{lag} = \frac{\left\{tan^{-1}\left[K_\phi\left(\frac{t-t_1}{t_{lag}}-1\right)\right]\right\}}{\pi} + \frac{1}{2} \tag{11}$$

$$t_{lag} = \frac{K}{\left(\frac{C_S^*}{C_M^*}\right)} \tag{12}$$

The specific decay rate for thick hyphae δ_T and thin hyphae is δ_H

$$\delta_T = 0.00441 \tag{13}$$

$$\delta_H = \frac{{\delta_H}^m}{1+I_1 S_1} \tag{14}$$

The product formation of CPC is

$$\frac{dP}{dt} = EX_T - \gamma P \tag{15}$$

The ϕ_{lag} function equation is exclusively designed to grow thin hyphae cells (X_H) entirely on glucose first until it is completely consumed, only after glucose is completely depleted – then sucrose is used. The ϕ_{lag} form is a smoothened step function, going from 0 at time t_1 (at which the time when glucose is fully consumed and the its concentration depletes to zero) to 1 at $(t_1 + t_{lag})$. For the simulation purpose, the theshold concentration of glucose is assumed to be 0.01 g/L and not 0 g/L, Therefore, after the glucose concentration decreases below 0.01 g/L, only then sucrose will start to be consumed.

The the symbols and the values of various constants are similar to those reported by Basak *et al.* [6]. The constants are given in Table 1, which correspond to fermentation experiments conducted at the temperature of 32 °C and pH 6.2.

2.2. Trial-and-error of feeding flowrate by step test

Numerous simulation runs are conducted to explore how different patterns of fed-batch feeding affect the desired product concentration. Thus, it is a trial and error approach to fed-batch feeding, i.e., by manipulating the feeding flow rate of either glucose or sucrose or both. The step input indicates the start of fed-batch mode and step output indicates the end of fed-batch mode. There are two manipulated variables which are the Step Time and the Feeding Flow rate. The feeding flow rate is limited to a range between 0.0 L/h and 0.5 L/h.

There are five scenarios of feeding profiles that are explored; an illustration is shown in Figure 1. The first scenario is a single step of sugar feeding which indicates a single fed-batch of either glucose or sucrose; the second scenario is a single step of sugar feeding with a constant small feeding flow rate of less than 0.05 L/h throughout the whole fermentation process; the third scenario is a double step feeding which indicates two fed-batch feeding of either glucose or sucrose; the fourth scenario is a double step of sugar feeding with a constant small feeding flow rate less than 0.05 L/h throughout the

whole process; and the fifth scenario consists of two single step of sugar feeding, which indicates a single fed-batch feeding of glucose and a single fed-batch feeding of sucrose in one fermentation process.

Table 1. Estimated values of parameters [6].

Parameter	Estimates	Unit
$\mu {S_1}^m$	0.04200	h^{-1}
$\mu {S_2}^m$	0.02100	h^{-1}
μT^m	0.04526	h^{-1}
mS_2	0.02267	h^{-1}
δ_T	0.00441	h^{-1}
${\delta_H}^m$	0.00668	h^{-1}
Y_{S1}	0.46188	
Y_{S2}	0.4	
I_1	20	g/L
I_2	300	g/L
K_{S1}	0.1	g/L
K_{S2}	10.1990	g/L
K	66.8	h
K_ϕ	318.2	
$\propto$	9	$(g/L)^{-1}\ h^{-1}$
β	2.18787	h^{-1}
γ	0.01076	h^{-1}
t_{lag}	34.7724	h^{-1}

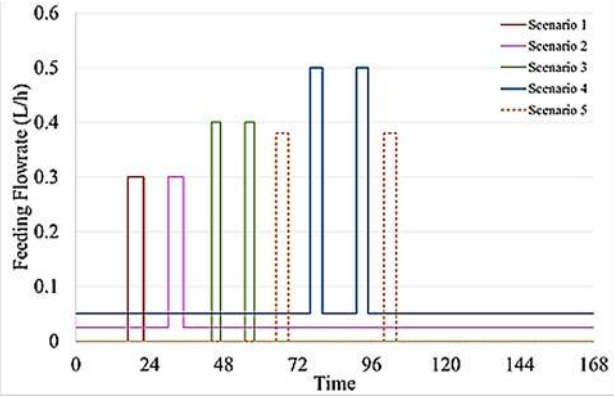

Figure 1. Illustration of the five feeding profile scenarios.

2.3. Principal Component Analysis

Principal Component Analysis (PCA) is conducted to find the optimal feeding strategy of CPC, i.e., the feeding strategy which results in the largest productivity. The PCA incorporated six variables, which includes the maximum cephalosporin concentration of (C_p), time taken to reach C_p (t_p), maximum thin cell concentration (X_h), time to reach Xh (t_h), maximum enzyme concentration (C_e), time to reach C_e (t_e), and liquid volume accumulated in the bioreactor (V_p) at $t = t_p$. These variables are chosen because from extensive simulation it is found that the maximum formation of thin cells and maximum production of enzyme strongly affect the maximum production of cephalosporin. The mass of CPC (M_p) and productivity of CPC (P_p) are then calculated to find the optimal feeding strategy. The related equations are as shown below.

The mass of CPC at the peak time (t_p) of fermentation is

$$M_p = C_p\, V_p \tag{16}$$

where C_p denotes the peak concentration of the CPC.

The productivity is calculated up to the peak time

$$P_p = M_p/t_p \quad (17)$$

The PCA is used to determine the extent of interactions among the six variables and their influences on the productivity of CPC fermentation.

2.4. Optimal Feeding Strategy Selection

The optimal feeding strategy is then selected from a set of trial-and-error runs by choosing the feeding strategy that produces the highest productivity of CPC, taking into account the time to reach the maximum peak concentration of CPC, and the final liquid volume in a 10-L bioreactor. It is desirable to fill up the bioreactor volume to about 90% in order to maximize its capacity.

3. Results and discussion

The software, MATLAB R2017b is used to simulate the batch model of Basak *et al*. (1995). Using the same software, the batch model is extended to the fed-batch simulations in order to find the optimal feeding strategy.This simulation is conducted to find a relation between behaviour of the *Acremonium Chrysogenum* cells during its consumption of glucose and sucrose as the carbon sources, and the development of thin cells into thick cells, towards the production of Cephalosporin, thus obtaining the maximum yield and productivity.

The initial values of four state variables in the simulation begin with a thin cell concentration of 5 g/L, glucose concentration of 35 g/L, sucrose concentration of 25 g/L, and 1-L of pre-culture containing seed and inoculum medium. The fermentation is simulated in a 10-L bioreactor. It is desired to obtain at least a final concentration of 1,000 mg/L of cephalosporin, and fill up to minimum 90% of the 10-L bioreactor in less than 168 hours.

3.1. Batch fermentation

The mathematical model (programed in Matlab code) is used to simulate a base case (batch fermentation) and the results are shown in Figures 2, 3 and 4. Note that, the results are compared with Basak *et al*. [6] to validate the reliability of the Matlab simulation. The glucose is consumed first till its depletion and only then the sucrose is consumed. This behavior is due to the fungus preference to use glucose as a primary carbon source to undergo morphological differentiation from thin cells to thick cells. The sucrose is used as a secondary carbon source in the subsequent stage for the growth of thick cells and production of cephalosporin. This behavior corroborates the *A. chrysogenum* morphology [7]. The thin cells (Xh) grow from an initial concentration of 5 g/L to reach a peak concentration of 20 g/L.

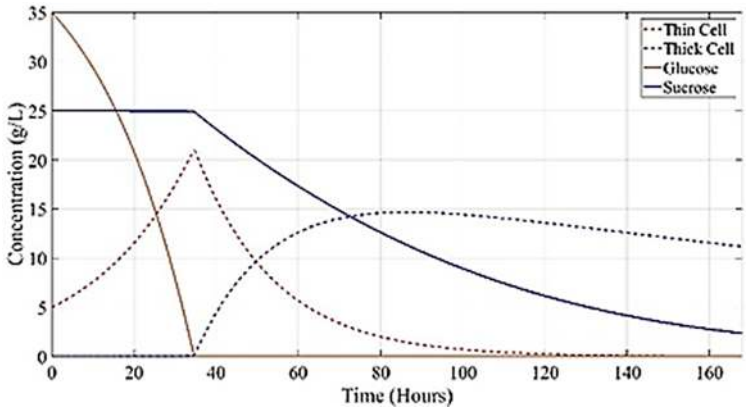

Figure 2. Batch fermentation of CPC: simulation profiles of thin cells, thick cells, and glucose and sucrose concentrations.

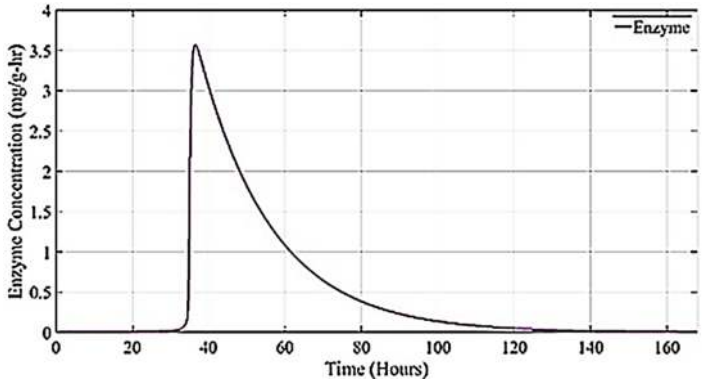

Figure 3. Batch fermentation of Cephalosporin C: simulation profile of enzyme concentration.

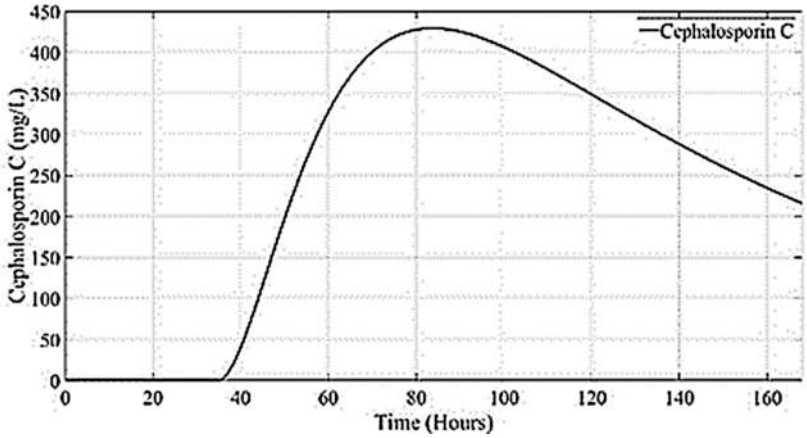

Figure 4. Batch fermentation of CPC: simulation profile of Cephalosporin concentration.

This thin cell growth happens as a result of the glucose consumption by the cells which reaches peak concentration when glucose is fully consumed. The thick cells (Xt) begin to grow only after glucose is fully depleted which marks the beginning of sucrose consumption. During this period, the enzyme concentraion increases exponentially to 3.5 mg/(g.h) and the cephalosporin production is initiated. It is observed that the maximum product concentration is reached when the thick cell concentration reaches its peak concentration, after which the thick cell concentration decreases. The maximum cephalosporin concentration is 430 mg/L at time 83.8 hours, with a productivity of 5 mg CPC/hour. The Matlab simulation results are very similar with the experimental results conducted by Basak *et al*. [6]; hence validating the Matlab model simulation.

3.2. Feeding profile scenarios – fed-batch fermentation

It is found that a strong relationship exists between the feeding flow rate with the production of Cephalosporin, based on numerous trial runs of changing the feeding flow rate by gradually increasing it from 0.1 L/h until 0.5 L/h. Results show that as the feeding flow rate increases, the product concentration decreases, but delays the initiation of Cephalosporin production, thus postpones the time for production to reach its maximum peak, which increases the local optimum productivity of CPC. This is because the faster the feeding rate, the longer it takes for the cells to consume the sugars, and the cephalosporin production decreases as fed-batch feeding is introduced [8]. The CPC production is stagnant while the cells are consuming the sugars, which means that the activation time to produce CPC and for its concentration to reach the maximum peak is delayed. The graph showing the effect of increasing the feeding flow rate and sugar consumption is shown in Figure 5.

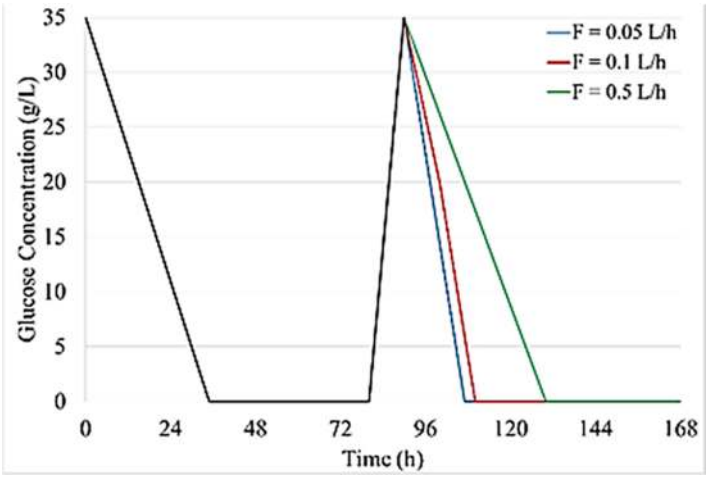

Figure 5. Illustration of the effect of different feeding flow rates on glucose consumption.

3.3. Factors affecting CPC production

A relationship between the CPC production and the initial values of two state variables, which are the concentration of glucose and sucrose is also studied. It is observed that by increasing the initial concentrations, it postpones the activation time of cephalosporin production. This is because as the initial substrate concentrations increase, the longer it takes for the cells to consume the sugars and this delays the differentiation from thin cell to thick cell, hence delaying the CPC production. Results show that the initiation to produce cephalosporin can be made faster with a lower initial glucose concentration, i.e., initial concentration of 35 g/L leads to aster initiation than that of 40 g/L. There are over 20 different feeding flowrate scenarios which are adopted in the simulation. Some of the feeding flowrate scenarios are shown in the Table 2.

Please note that, the timing of introducing the fed-batch feeding is also studied. Results show that the effects of timing of introducing the fed-batch feeding of glucose and sucrose are quite different. When glucose fed-batch feeding is introduced right after the initial glucose is fully consumed, more CPC is produced. But when glucose fed-batch feeding is introduced in a later time, less cephalosporin is produced instead. This is because when the thin cells are fed with glucose right after the initial glucose is fully consumed, the thin cells keep on growing from the initial glucose consumption, which as a result produces a larger amount of thick cells after cell differentiation, hence producing more Cephalosporin at the end of the fed-batch [4]. On the other hand, with sucrose fed-batch feeding, a relatively small amount of cephalosporin as the sucrose is introduced at any given time. This is because of the fungus only requires sucrose as a secondary carbon source for maintenance of the existing cells if needed, unlike glucose as a primary carbon source for cell growth.

Moreover, the step time interval of fed-batch feeding is also studied, in which several changes in the step time interval of fed-batch feeding of glucose only, and fed-batch feeding of sucrose only show the same production behaviour. Results show that a smaller step time produces more cephalosporin but fills up less of the bioreactor volume, a 10-hours step time of fed-batch feeding produces higher cephalosporin final concentration than that of a 20-hours step time. This is because the longer the step time, the longer time it takes for the cells to consume the sugars and during consumption time, there is no production of cephalosporin, hence less final concentration of cephalosporin can be achieved [7].

In addition to studying the above parameters, the fed-batch feeding scenario also affects the CPC production. Results in Table 2 show that the glucose feeding only can produce the large amount of CPC but vice versa under the sucrose feeding only, which leads to the least amount of cephalosporin. The summarized results of the various feeding scenarios are shown in Table 2. With the glucose fed-batch feeding, the multiple steps of fed-batch feeding flowrate (from single step to double step) gives more cephalosporin at the end of fermentation. The reason for this is that the feeding of glucose can increase the thin cell concentration, which increases the enzyme production, and in turn this produces a larger

cell differentiation from thin cells to thick cells. Consequently, the multiple step feeding strategy produces more cephalosporin. On the contrary, with sucrose fed-batch feeding, it can be seen that the opposite behaviour occurs. This is because sucrose is only essential for cell maintenance and not for cell growth. Hence, adding more fed-batch feeding of sucrose to the process tends to decrease the cephalosporin production as the presence of additional sucrose supresses the thin cell growth and decreases the enzyme concentration, thus the cephalosporin production is reduced.

Table 2. Feeding flowrate scenarios and the results.

No.	Feeding Flowrate Scenario	X_h (g/L)	t_h (h)	C_e (mg/(g.h))	t_e (h)	C_p (mg/L)	t_p (h)	V_p (L)	M_p (mg)	P_p (mg/h)
1	Scenario 4A (Glucose)	35.3	98	5.53	100.15	1053	145	9.0	9477.0	65.36
2	Scenario 4B (Glucose)	30.0	109	4.72	110.6	1072	151	9.0	9673.7	64.06
3	Scenario 3 (Glucose)	24.7	79.2	3.69	81.0	423.5	124.5	7.4	3118.7	25.05
4	Scenario 2 (Glucose)	28.0	68	3.50	70.5	641.5	138.0	5.4	3441.0	24.93
5	Scenario 1 (Glucose)	23.0	57.5	2.44	59.5	625.5	148.0	5.4	3355.2	22.67
6	Scenario 5 (Glucose and Sucrose)	13.0	35	2.00	36.8	56	50.0	9.0	505.3	10.11
7	Scenario 1 (Sucrose)	12.0	35	2.05	36.6	104	100.0	6.9	713.4	7.13
8	Scenario 2 (Sucrose)	12.0	35	2.00	36.6	108.55	100.0	4.9	527.6	5.28
9	Scenario 3 (Sucrose)	12.28	34.7	2.05	36.6143	110.5	112.0	4.9	537.0	4.79
10	Scenario 4 (Sucrose)	8.0	35	1.30	36.9	43.0	100.0	9.6	411.1	4.11

3.4. Optimal feeding strategy of fed-batch fermentation

From Table 2, the Scenarios 4 of glucose feeding only are considered desirable as these produce high peak CPC concentration above 1000 mg/L. This high final concentration is achieved with the feeding flowrate profile shown in Figure 6. The glucose feeding flowrate profile is adjusted by taking into account all of the feeding flowrate factors affecting the cephalosporin production through numerous simulation trials and PCA. The Scenario 4A produces the highest productivity of CPC equals to 65.36 mg/h with the final maximum cephalosporin concentration of 1053 mg/L. Thus, from the set of the trial-and-error runs, this is considered the optimal feeding strategy in this study. The feeding profile shown in Figure 6 employs a small continuous feeding flow rate of 0.018 L/h in addition to the two large pulse fed-batch feeding flowrates of 0.5 L/hr for 5 hours duration each.

The first large pulse feeding is introduced at time 50 hours and stopped at time 55 hour while another pulse at time 80 to 85 hour. These exact times are chosen because as shown in Figure 7, the glucose concentration decreases gradually at hour 50 which produces a thin cell of 21 g/L. Before glucose is fully consumed, the first pulse feeding of glucose containing 34 g/L is introduced and this leads to the thin cell concentration rises to the second peak of 36 g/L. After glucose is fully consumed at time 80 hours, a second pulse feeding of glucose with a concentration of 33 g/L is again introduced, and subsequently this produces the third peak of thin cell with concentration of 35 g/L and enzyme concentration of 3.8 mg/(g.h) as shown in Figure 8.

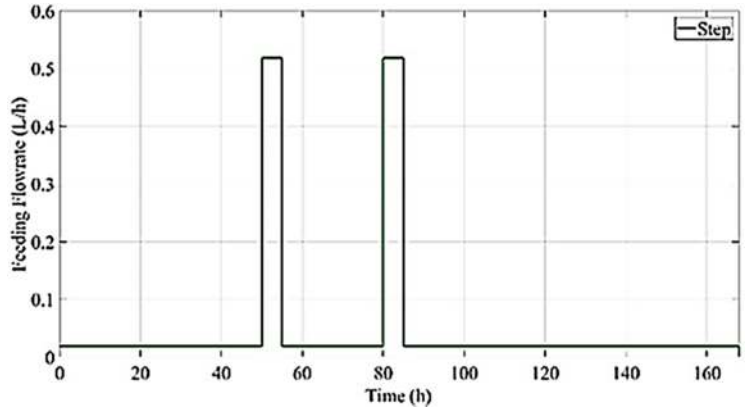

Figure 6. Fed-batch scenario 4A glucose only: plot of step time feeding profile over time.

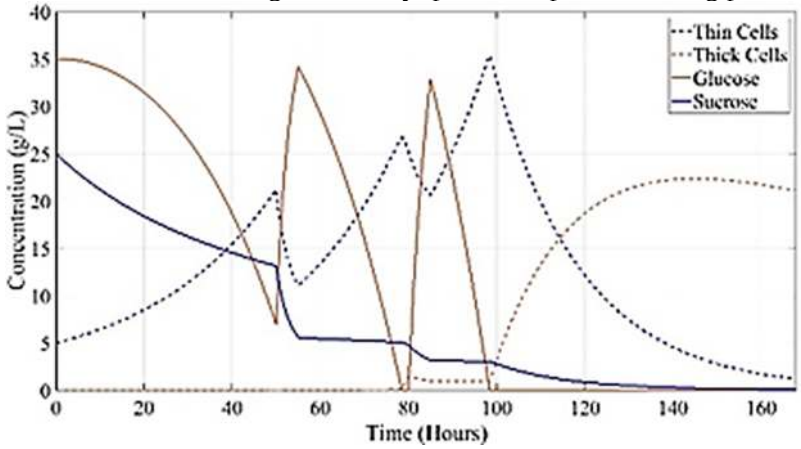

Figure 7. Fed-batch scenario 4A glucose: plot of thin cells, thick cells, glucose and sucrose concentration over time.

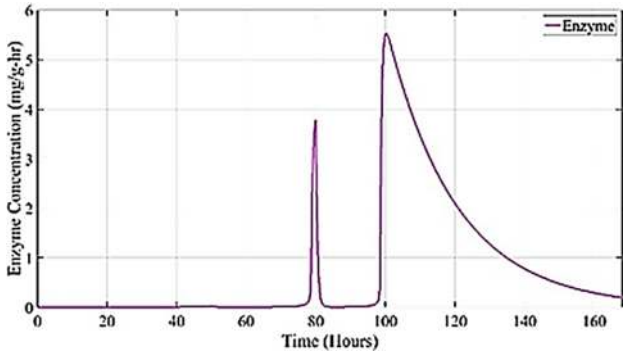

Figure 8. Fed-batch scenario 4A glucose: plot of enzyme concentration over time.

Thin cells fully consume the glucose until time 100 hour. After this time the thick cells start to be produced the production of CPC begin thereafter. The maximum peak of cephalosporin production is achieved at time 145 hour, and at the same time the thick cells reach its peak of 23 g/L. At the end of the fermentation process (after 168 hours), the bioreactor volume fills up to 90% of the 10-L bioreactor. A total of 9,477 mg of CPC is produced giving the productivity of 65.36 mg-CPC/h.

4. Conclusion

In the present work, an optimal feeding strategy for the fed-batch fermentation of cephalosporin has been obtained. This optimal feeding strategy leads to a peak concentration of cephalosporin of 1,053 mg/L correspoding to a productivity of 65.36 mg/hr. So far, the present value represents the highest final

concentration of cephalosporin achieveble. Extensive numerical studies indicate that the major factors affecting the cephalosporin production are the initial glucose and sucrose concentrations, feeding flow rate, time and duration of feeding, and feeding scheme (either glucose or sucrose or both). The direction for future works will be to extend the current study towards feeding strategy based on automatic control algorithm implemented using a double-loop or triple-loop multi-scale control structure [9]. This control algoirthm could be effective as it can handle complex unstable systems, such as the fed-batch fermentation. Furthermore, the multi-scale model based optimization approach will also be worth exploring in futute works, e.g., the multi-scale optimization method of [10].

Notations

Symbol	Definition	Unit
C_S^*	sucrose concentration at time at the point of glucose depletion	g/L
C_M^*	total cell mass concentration at time at the point of glucose depletion	g/L
E	Concentration of enzyme inside the thick wall cells	$mg/(g.h)$
F	Inoculum Medium	g/L
P	Concentration of Cephalosporin C	(mg/L)
S_1	Glucose Concentration	g/L
S_2	Sucrose Concentration	g/L
X	Total cell mass (biomass accumulation)	g/L
X_H	Thin hyphae cells	g/L
X_T	Thick walled cells	g/L
$\boldsymbol{K_{S1}}$	Saturation constant related to glucose	g/L
$\boldsymbol{K_{S2}}$	Saturation constant related to sucrose	g/L
$\boldsymbol{K}$	constant in proposed lag time expression	h
$\boldsymbol{K_\phi}$	constant related to the steepness of the arctangent curve rising from zero	-
$\boldsymbol{t_{lag}}$	Lag period	h^{-1}
$\boldsymbol{I_1}$	Repression constant of morphological differentiation by glucose	g/L
$\boldsymbol{I_2}$	Inhibition constant of sucrose use by glucose	g/L
$\boldsymbol{mS_2}$	Maintenance constant	h^{-1}
$\boldsymbol{Y_{S1}}$	Cell mass yield factor from glucose	-
$\boldsymbol{Y_{S2}}$	Cell mass yield factor from sucrose	-
$\propto$	Growth-link enzyme formation rate	$(g/L)^{-1}\ h^{-1}$
$\boldsymbol{\beta}$	decomposition rate of the growth-link enzyme	h^{-1}
$\boldsymbol{\gamma}$	Cephalosporin C decomposition rate constant	h^{-1}
$\boldsymbol{\mu {S_1}^m}$	maximum specific growth rate of thin hyphae on sucrose	h^{-1}
$\boldsymbol{\mu {S_2}^m}$	specific growth rate of thin hyphae on sucrose	h^{-1}
$\boldsymbol{\mu T^m}$	maximum specific formation rate of thick wall cells	h^{-1}
$\boldsymbol{\mu_H}$	specific formation rate of thin hyphae (X_H)	h^{-1}
$\boldsymbol{\mu_T}$	specific formation rate of thick wall cells (X_T)	h^{-1}
$\boldsymbol{\mu_{S1}}$	specific growth rate of thin hyphae on glucose	h^{-1}
$\boldsymbol{\mu_{S2}}$	specific growth rate of thin hyphae on sucrose	h^{-1}
$\boldsymbol{\delta_T}$	specific decay rate of thick wall cells	h^{-1}
$\boldsymbol{{\delta_H}^m}$	maximum specific decay rate of thin hyphae	h^{-1}
$\boldsymbol{\delta_H}$	specific decay rate of thin hyphae	h^{-1}
$\boldsymbol{\delta_T}$	specific decay rate of thick wall cells	h^{-1}
$\boldsymbol{\phi_{lag}}$	intermittent lag function	-

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