Enhancement of PHB Production Process in a Fed-Batch Bioreactor Using Input-Output Linearization Technique with Optimal Setpoints

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Research Article

Enhancement of PHB Production Process in a Fed-Batch Bioreactor Using Input-Output Linearization Technique with Optimal Setpoints

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Abstract

Polyhydroxy butyrate (PHB) is a polyester that has been widely applied to formulate bioplastics. A challenge for the PHB production is the enhancement of PHB accumulation in bacterial cells, which can be conducted through the nutrient feeding strategy. A control system based on an input/output (I/O) linearization technique for the PHB production in a fed-batch bioreactor is proposed in this work. The concept of feast/famine phase is employed to provide the optimal desired targets for each time instant through optimization problems. The developed controller is applied to regulate the controlled output to follow the desired setpoints by manipulating the feed flow rate of nutrients. Simulation results show that the proposed control system attempts to follow the optimal desired targets (discrete-time approach) during the process operation. The accumulation of PHB in bacterial cells is around 25% higher than that of the process with a modified PI controller. Control performance tests indicate that the proposed control strategy successfully regulates the process according to the desired targets and handles the process disturbance effectively.

Keywords: Polyhydroxy butyrate (PHB), Bioprocess, Bioreactors, Optimization, Process control

1 Introduction

Polyhydroxyalkanoates (PHAs) are a group of bio-based polyesters synthesized by microorganisms which are fully biodegradable, biocompatible and present thermoplastic properties similar to those of conventional polyolefins such polyethylene (PE) or polypropylene (PP). Among the PHAs derivatives, polyhydroxybutyrates (PHBs) have been extensively studied and applied for various application such as packaging films, bags, containers, medicines, insecticides, herbicides, etc. [1]. The PHB can be synthesized by various types of bacteria and accumulated as energy storage granules in the cytoplasm of the cells. The previous studies demonstrated that the accumulation of PHB is depended on the operating conditions and media compositions of the bioreactor. In order to increase the intracellular PHB accumulation, the switch periods of

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the external substrate available (Feast) and unavailable (Famine) under aerobic conditions are studied to promote the intracellular PHB accumulation [2]. An optimal nutrient composition can be applied for the cultivation in a batch reactor and adjusted dynamically in the fed-batch reactor through feeding strategies. For the production in a fed-batch process, the system efficiency is generally depended on control techniques that are applied to calculate the nutrient feeding. Advanced control techniques have been applied in some works to handle the process fluctuation or uncertain reaction rates [3]. Since the PHB is accumulated intracellularly, on-line monitoring system may be unavailable for the real time operation. Thus dynamic models have been applied to predict the process behaviour and improve the controller efficiency.

There are some research works that developed control systems for the fed-batch reactor for chemical and biochemical processes with nonlinear behavior. Cougnon *et al.* [4] proposed an adaptive extremum seeking control for the fed-batch reactor which results show that the controller can regulate the process to the desired setpoints. Titica *et al.* [5] presented an adaptive extremum seeking control of the fed-batch reactor with Haldane kinetic which results show that an algorithm can drive the process to desired setpoints. Wu *et al.* [6] proposed the optimal adaptive control to find the optimal feeding strategy for the PHB production process in the fed-batch reactor. Cornet *et al.* [7] presented an adaptive linearizing inferential control to regulate the ammonium concentration during the growth phase of the PHB production in a fed-batch reactor which results shows that the controller has been successful to regulate the process for a fed-batch reactor. As mentioned about the control system for the fed-batch reactor, most of the previous works investigated control systems by using a set of optimal setpoint which may have a limitation for real-time operation that requires updated setpoints. Input/output (I/O) linearization technique has been applied for the nonlinear system in some works [8], [9]. An important benefit of the I/O linearization technique is the ability to manipulate the system rapidly, which can be potentially applied to the process with updated setpoint.

In this work, the optimal setpoint for the process is updated in each time instant and the I/O linearization controller is employed to design a control system that compute the manipulated input of the process. To enhance the intracellular PHB accumulation, the optimal carbon to nitrogen (C/N) ratio in discrete-time system is applied to maximize the PHB accumulation for each time step. The main objective of this work is to develop a control system based on the I/O linearization technique that applies updated optimal setpoints obtained from optimization problems. The concept of nutrient limitation technique (feast and famine) is used to formulate the optimization problems combined to the developed control structure for maximization of cell concentration and intracellular PHB accumulation through the nutrient feed flow rate.

2 Materials and Methods

2.1 *Problem formulation*

A set of the mathematical model shown in Equation (1) represents the general form of the dynamical model for the PHB fermentation process. The state variables are written in the form as [Equations (1) and (2)]:

$$\frac{d\boldsymbol{\xi}}{dt} = \boldsymbol{F}_{in}\boldsymbol{\xi}_{in} - D\boldsymbol{\xi} + \boldsymbol{K}\boldsymbol{r}\left(\boldsymbol{\xi}\right) \tag{1}$$

$$y = h\left(\boldsymbol{\xi}\right) \tag{2}$$

with initial conditions [Equation (3)]:

$$\boldsymbol{\xi}^{0}(\mathbf{0}) = \boldsymbol{\xi}_{initial} \tag{3}$$

where the state variables and parameters of the PHB fermentation process are shown as:

$$\xi = \begin{bmatrix} X_R \\ P \\ N \\ S \end{bmatrix}, r(\xi) = \begin{bmatrix} \mu(\xi) X_R \\ X_R \end{bmatrix}, K = \begin{bmatrix} 1 & 0 \\ k_1 & k_2 \\ -k_3 & 0 \\ -k_4 & -k_5 \end{bmatrix}$$

$$D = \frac{F_1 + F_2}{V}, \boldsymbol{F}_{in} = \begin{bmatrix} 0 \\ 0 \\ F_2 \\ F_1 \end{bmatrix} \text{ and } \boldsymbol{\xi}_{in} = \begin{bmatrix} 0 \\ 0 \\ N_0 \\ S_0 \end{bmatrix}$$

where the vector of state variables is shown by $\boldsymbol{\xi}$, $\boldsymbol{\xi}_{in}$ is the vector of state variables of the inlet stream of a fed-batch reactor and $\boldsymbol{D}$ is the dilution rate. $\boldsymbol{F}_{in}$ is the feed inlet of the reactor; $\boldsymbol{K}$ is the vector of the yield for

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the substrate consumption or product formation. $\boldsymbol{r}(\boldsymbol{\xi})$ is the reaction rate in the reactor, $\boldsymbol{y} = [y_1, ..., y_m]^T$ is the vector of controlled outputs, $t \in [0, \infty]$ is the time and h is the nonlinear function. The dynamical model in Equation (1) is applied to study the process behavior and formulate an advanced control scheme for the fed-batch reactor.

2.2 *Input/output (I/O) linearization control technique*

The function of nonlinear system can be written as the follow:

$$\dot{\boldsymbol{\xi}} = f(\boldsymbol{\xi}, u), \qquad y = h(\boldsymbol{\xi}) \tag{4}$$

where u is a vector of manipulated variables, y is a vector of controlled output variables and f is a vector of the nonlinear functions. The closed-loop responses of the process variables are shown in the following linear form as:

$$(\beta_i D + 1)^{r_i} y = v(t) \tag{5}$$

where D is the differential operator (i.e. $D = d/dt$), $v(t)$ is the compensated setpoint vector which can be updated in each time instant and β_i is the tuning parameter used to adjust the speed of the output response. The relative order which is shown in Equation (5) of the nonlinear process system is presented by $r_1, \ldots, r_{m-1}, r_m$ where r_i is the smallest integer that $\partial\left[d^{r_i} y_i / dt^{r_i}\right] / \partial u \neq 0$ as shown by the following equation [8]:

$$\begin{aligned}
y_i &= h(\xi) \\
\frac{dy_i}{dt} &= \left[\frac{\partial h}{\partial \xi}\frac{\partial \xi}{\partial t}\right] = h^1(\xi) \\
\frac{d^2 y_i}{dt^2} &= \left[\frac{\partial h^1}{\partial \xi}\frac{\partial \xi}{\partial t}\right] = h^2(\xi) \\
&\vdots \\
\frac{d^{r-1} y_i}{dt^{r-1}} &= \left[\frac{\partial h^{r-2}}{\partial \xi}\frac{\partial \xi}{\partial t}\right] = h^{r-1}(\xi) \\
\frac{d^r y_i}{dt^r} &= \left[\frac{\partial h^{r-1}}{\partial \xi}\frac{\partial \xi}{\partial t}\right] = h^r(\xi, u)
\end{aligned} \tag{6}$$

In order to formulate the control system for the PHB fermentation process, Equations (4) and (5) are applied. The manipulated input $u(t)$ can be shown in the compact form as [Equation (7)]:

$$u(t) = \psi\left(\bar{\xi}(t), v_i(t)\right) \tag{7}$$

2.3 *Process description*

To enhance the PHB production in the fed-batch bioreactor, the concept of the two-step fermentation strategy can be applied to promote the PHB concentration and maximize the cell concentration [10]–[12].

2.3.1 Feast phase

The carbon and nitrogen source are the essential nutrients for cell growth and intracellular accumulation of PHB in the fed-batch bioreactor. The feast phase exists when the carbon and nitrogen source are presented or fed to the reactor. In this condition, bacterial cells can optionally produce the biomass or intracellular PHB. The lack of nitrogen source or nitrogen depletion for a moment can make a decrease in the number of intracellular enzymes for cell growth. During the nitrogen source depletion, the bacterial cells can accumulate the PHB instead of the biomass production.

2.3.2 Famine phase

The feast phase indicates that both carbon and nitrogen source are important for the accumulation of PHB in microbial cells. The famine phase exists when the carbon source depletion and nitrogen source is presented. In the famine phase, the intracellular PHB is consumed for cell growth and maintenance.

Due to the importance of feast and famine phase, the feeding strategy of nutrients in the fed-batch bioreactor can affect the final intracellular accumulation of PHB. Since the fed-batch bioreactor allow us to adjust the nutrient concentration during the operation, the setpoint of the closed-loop system can be optimized to enhance the product formulation. A simplified fed-batch bioreactor for the PHB production process can be shown in Figure 1.

2.4 *Mathematical model of fed-batch PHB fermentation process*

To develop a model-based control system for the PHB

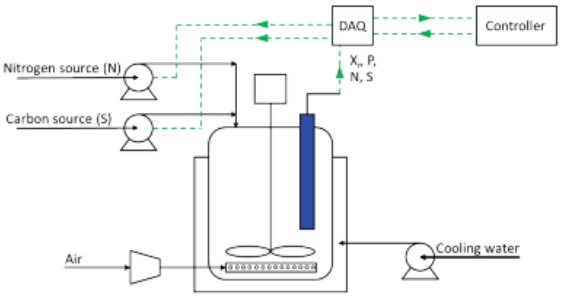

Figure 1: A diagram of the PHB fermentation process in the fed-batch bioreactor.

fermentation process, a reliable model is determined to formulate the controller.

The process model for the PHB production by using Ralstonia eutropha in a fed-batch bioreactor was developed and validated in previous works [11], [13], [14]. The unstructured-non segregated model is simulated to investigate responses of the process in this work. The mathematical model for the fed-batch PHB fermentation process can be shown as:

$$\frac{dX_r}{dt} = \mu X_r - \frac{(F_1 + F_2)}{V} X_r \tag{8}$$

$$\frac{dP}{dt} = (k_1 \mu X_r) + (k_2 X_r) - \frac{(F_1 + F_2)}{V} P \tag{9}$$

$$\frac{dN}{dt} = -k_3 \mu X_r - \frac{(F_1 + F_2)}{V} N + \frac{F_2}{V} N_{in} \tag{10}$$

$$\frac{dS}{dt} = -k_4 \mu X_r - k_5 X_r - \frac{(F_1 + F_2)}{V} S + \frac{F_1}{V} S_{in} \tag{11}$$

$$\frac{dV}{dt} = F_1 + F_2 \tag{12}$$

where X_r is the residual biomass concentration, P is PHB concentration accumulated in the microbial cells, N and S are nitrogen and carbon concentration respectively and V is the operating volume. F_1 and F_2 are the feed flow rate of carbon and nitrogen sources to the reactor, respectively. S_{in} and N_{in} denote the nutrient concentrations of carbon and nitrogen source in the feed stream. The specific growth rate of bacteria (μ) can be presented in Equation (13), the equation shows that μ relates to the ratio of carbon and nitrogen.

$$\mu = \mu_m * \left(\frac{\left(\frac{N}{S}\right)}{\left(\frac{N}{S}\right) + K_{sr}} \right) * \left(1 - \left(\frac{\frac{N}{S}}{S_m} \right)^{n_k} \right) \tag{13}$$

The parameters for PHB fermentation process can be shown in Table 1. Consider the mathematical model for intracellular PHB production in Equation (9), there are two main parts have been focused. The growth-associated part relates to the growth of bacteria and the non-growth-associated part only relates to the biomass. The Equations (8)–(12) show the dynamics of the biomass concentration, the PHB concentration, the nitrogen source concentration, the carbon source concentration and reactor volume, respectively. In this case, the residual biomass (X_r) can be obtained from the difference between total biomass concentration (X) and intracellular PHB concentration (P) in cells as the following Equation (14).

$$X = X_r + P \tag{14}$$

Table 1: The parameters for the PHB fermentation process [3]

Symbol	Description	Value	Unit
μ_m	Maximum specific growth rate	0.437	h^{-1}
K_{sr}	Monod constant for cell growth	0.0697	g/L
S_m	Value of carbon/nitrogen ratio when $\mu = 0$	0.073	-
k_1	Kinetic constant	0.3	-
k_2	Kinetic constant	0.002	h^{-1}
k_3	Kinetic constant	0.08	-
k_4	Kinetic constant	1.831	-
k_5	Kinetic constant	0.067	h^{-1}
S_{in}	Carbon source concentration in feed	125	g/L
N_{in}	Nitrogen source concentration in feed	28	g/L

2.5 *Formulation of the control system for PHB fermentation process*

In this study, the dynamical models of the PHB production in a pilot-scale process is determined for the simulation. Since the intracellular PHB accumulation relates to the carbon and nitrogen source, the nutrients are used to manipulate the system. The developed control system should have ability to handle

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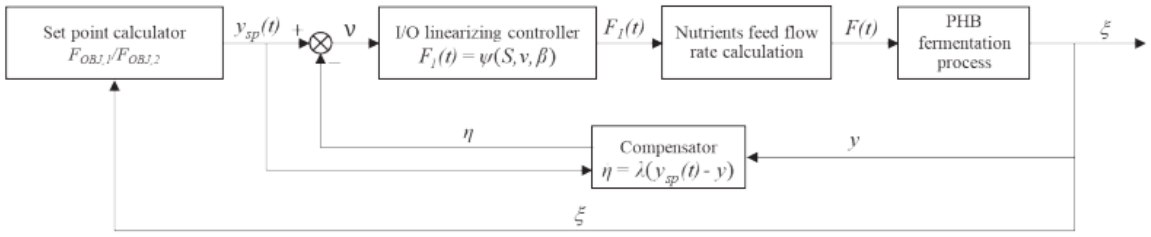

Figure 2: Picture scanned into the paper across two columns.

the process fluctuation from nutrients feeding, growth of bacterial culture and unknown disturbances that can possibly occur during the process operation. The control structure of the developed control scheme can be shown in Figure 2.

2.5.1 Input/output (I/O) linearization control with an integral action for PHB fermentation process

The I/O linearization control technique is applied to formulate the controller that can force the system to follow the updated targets. The desired targets are computed by applying optimization problems for maximization of the cell growth rate and intracellular PHB accumulation. In order to develop the control system, the I/O linearization technique from Equation (5) and the dynamics of the carbon source concentration in Equation (11) are applied to formulate the equation system for the optimal nutrients feed setpoints. The I/O linearizing controller can be written as [Equation (15)]:

$$(\beta D + I)^{r_i} S(t) = v(t) \tag{15}$$

The dynamics of the carbon source in Equation (11) and the relative order (r_i) definition of Equation (6) was substituted to Equation (5), and then the equation is rearranged to obtain the feed flow rate of the carbon source. The formulated manipulated input equation based on I/O linearization control can be shown as:

$$F_1(t) = \frac{V(t)}{S_{in} - S(t)}\left[\left(\frac{v(t) - S(t)}{\beta}\right) + k_4 \mu X_r + k_5 x_r + \frac{F_2}{V} S(t)\right] \tag{16}$$

where β is the tuning parameter for I/O linearization control and $v(t)$ is the output set points which is the function of the time dependent. The $v(t)$ is shown as [Equation (17)]:

$$v(t) = y_{sp}(t) - \eta(t) \tag{17}$$

where $\eta(t)$ is an integral action. $y_{sp}(t)$ is the setpoint in each time step. The integral action is combined to the control system in Equation (16) as [Equations (18) and (19)]:

$$\dot{\eta}(t) = \lambda E(t) \tag{18}$$

$$E(t) = \left(y_{sp}(t) - S(t)\right) \tag{19}$$

where $\eta(t)$ is an integral error from the control output, λ is the tuning parameter for an integral action term and $E(t)$ is the tracking error. For the integral action, Equation (18) is solved to obtain $\eta(t)$ as follows [Equation (20)]:

$$\eta(t) = \lambda \int_0^t E(t)\, dt \tag{20}$$

2.5.2 Optimization problem for PHB fermentation process

The main objective of the optimization problem for the PHB fermentation process is to calculate the optimal carbon source consumption for maximizing the intracellular PHB accumulation by the concept of the feast and famine phase. This optimization problem is formulated based on the concept. The objective functions are solved to provide the optimal setpoint that promote the growth rate of cells and intracellular PHB accumulation.

The objective function $F_{OBJ,1}$ is proposed to compute the optimal nutrients feed flow for cell growth rate, it can be shown as [Equation (21)]:

$$F_{OBJ,1} = \mu\left(S, N, F_1, F_2\right) \tag{21}$$

where $\mu\left(S, N, F_1, F_2\right)$ is the specific growth rate that

is related to the carbon source, nitrogen source and nutrients feed flow rate. The aim of the objective function $F_{OBJ,1}$ is to find the set point to be tracked by the control action of Equation (16) in order to obtain the optimal nutrients feed flow that maximizing the cell growth rate. After that, the objective function will be switched from $F_{OBJ,1}$ to $F_{OBJ,2}$ for increasing the intracellular PHB accumulation.

The objective function $F_{OBJ,2}$ which used to find the optimal nutrients feed flow for maximizing the intracellular PHB accumulation. The objective function $F_{OBJ,2}$ can be shown as [Equation (22)]:

$$F_{OBJ,2} = P\left(S,N,F_1,F_2,\mu,t\right) \tag{22}$$

where $P\left(S,N,F_1,F_2,\mu,t\right)$ is the intracellular PHB concentration, which is related to the carbon and nitrogen source, nutrients feeding, specific growth rate and time. The aim of the objective function $F_{OBJ,2}$ is to find the optimal nutrients flow rate for each time instant that maximize the intracellular PHB accumulation. To achieve that, the dynamical equation of PHB concentration in Equation (9) is applied to formulated the $F_{OBJ,2}$ which can be shown in Equation (23), and then nutrients feed flow rate will be obtained.

$$P\left(S,N,F_1,F_2,\mu,t\right) = \int \frac{dP(t)}{dt}\,dt \tag{23}$$

In order to apply the proposed control strategy for the PHB fermentation process, the process variables are assumed to be estimated by the reliable equipment. The objective function $F_{OBJ,1}$ and $F_{OBJ,2}$ for the optimization problem with constrained are solved at each time instant for calculating the optimal nutrients feed flow rate, which can be written as [Equations (24) and (25)]:

$$\min_{R} -F_{OBJ,1} \;\; and \;\; \min_{F_2} -F_{OBJ,2} \tag{24}$$

$$F_{\min} \le F(t) \le F_{\max} \tag{25}$$

$$R_{\min} \le R \le R_{\max} \tag{26}$$

The available range of the nutrients feed flow rate and carbon/nitrogen ratio of the fermenter is limited as Equations (25) and (26), respectively. The PHB fermentation process initially starts with a concept of batch operation, and then the nutrients are fed to the fed-batch reactor. The operation time at the beginning of the process is shown as t_b. For the fed-batch operation, the $F_{OBJ,1}$ is computed to obtain the optimal carbon and nitrogen concentration ratio (R) that gives the maximum rate of cell growth. After that, the optimal setpoint $y_{sp}(t)$ can be obtained in each time instant by solving $F_{OBJ,1}$ during the process operation to maximize the cell concentration. The carbon-source feed rate (F_1) is computed from the developed I/O linearization-based controller of Equation (16) while the nitrogen source feed rate (F_2) is fed according to the same ratio R between F_1 and F_2. The operation time for $F_{OBJ,1}$ is shown by t_{WPtrh}. W_P is defined as the mass fraction of PHB content in the total biomass which can be presented as [Equation (27)]:

$$W_p = \frac{P(t)}{X(t)} \tag{27}$$

When W_P reaches the threshold, the objective the function will be changed from $F_{OBJ,1}$ to $F_{OBJ,2}$, the condition can be written as [Equation (28)]:

$$F_{OBJ} = \begin{cases} F_{OBJ,1}\,, W_p < W_P^{trh} \\ F_{OBJ,2}\,, W_p \ge W_P^{trh} \end{cases} \tag{28}$$

where $F_{OBJ,1}$ is switched, the $F_{OBJ,2}$ is solved instead of $F_{OBJ,1}$ to obtain the optimal nitrogen source feed flow rate which makes the highest intracellular PHB accumulation and the carbon source feed flow rate is obtained by Equation (16). A flowchart that illustrates the proposed control strategy can be shown in the Figure 3. In order to investigate the responses of the proposed control system, the computational programming such MATLAB is employed.

3 Results and Discussion

The proposed control strategy is applied to regulate the PHB fermentation process by using molasses-vinasses as the carbon source. The detected and estimated state variables are sent to the controller for computing the optimal nutrients feed rate with the constrained discrete-time approach. For this section, the results of the PHB fermentation process under the fed-batch fermentation are demonstrated.

3.1 *Closed-loop responses*

The simulation results of the proposed control strategies

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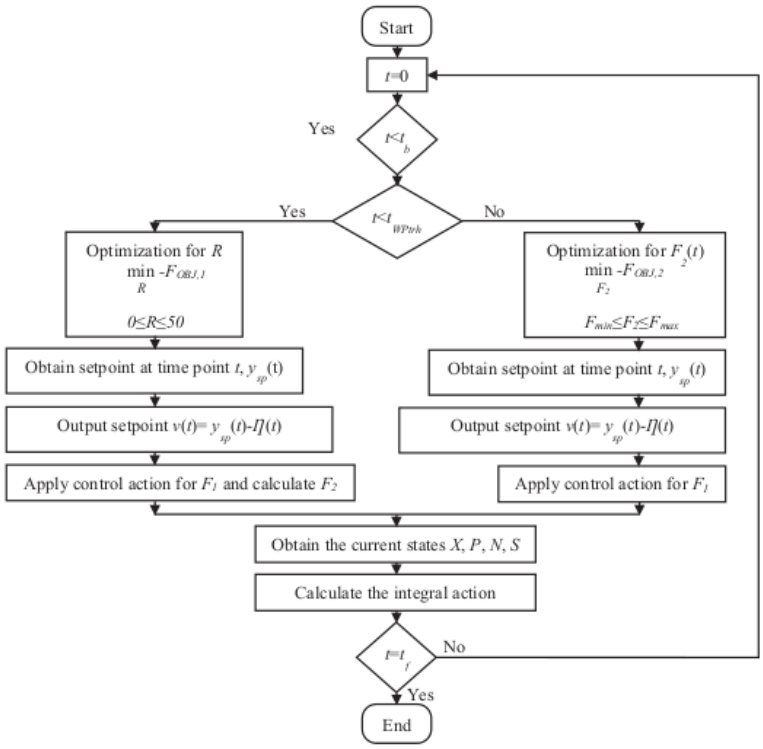

Figure 3: The flowchart for summary the process calculation of the nutrients feeding strategies through the I/O linearization control system.

(I/O linearization control) with optimization problems is used to regulate the nutrients concentration in the reactor to follow the desired targets that enhance the intracellular PHB accumulation. The initial conditions for the PHB fermentation process in the fed-batch reactor and tuning parameters for control systems are shown in Table 2. The process is initially operated with the concept of a batch fermentation, then the proposed control system is applied to regulate the fed-batch fermentation. During the fed-batch operation, the I/O controller applies the updated setpoints, obtained from the objective functions $F_{obj,1}$, and $F_{obj,2}$, to achieve the optimal nutrients feeding that maximize PHB accumulation of the bacterial cells. The simulation results for optimization problems can be shown in Figure 4. The figure shows that the total biomass and intracellular PHB concentration after 30 h of operation can be obtained by 32 g/L and 11g/L, respectively. The results of the controlled output in Figure 4(b) show that the proposed control strategy attempt to follow optimal setpoints of each time step. A modified PI controller that applied the updated setpoints is performed to control the process system. A comparison of the proposed control strategy with the PI controller shows a better performance to follow the updated setpoints of the developed control system.

Table 2: The initial conditions and tuning parameters for the PHB fermentation process

Symbol	Description	Value	Unit
X_{r0}	Initial residual biomass concentration	3	g/L
P_0	Initial PHB concentration	0	g/L
N_0	Initial nitrogen source concentration	1.5	g/L
S_0	Initial carbon source (total sugar) concentration	30	g/L
V_0	Initial volume	50	g/L
β	Tuning parameter for I/O controller	0.8	h
λ	A tuning parameter for I/O controller	0.0048	1/h
k_p	A tuning parameter for PI controller (the proportional gain)	0.7	-
k_i	A tuning parameter for PI controller (the integral gain)	0.006	-

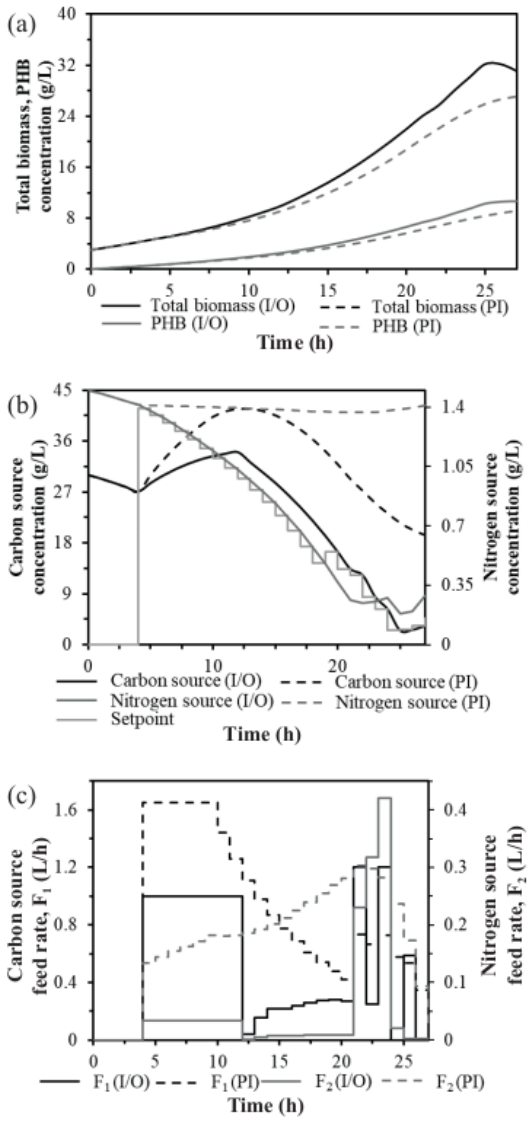

Figure 4: Closed-loop responses for proposed control strategies (a) total biomass and intracellular PHB accumulation (b) carbon and nitrogen source concentration (c) nutrients feed rate.

For the nitrogen source feeding, it is clear from the result that the proposed controller regulates the nitrogen feed flow rate corresponding to the manipulated input while the nitrogen concentration controlled by the PI controller is excess. The accumulation of PHB in bacterial cells is around 25% higher than that of the process with a modified PID controller.

When compare the simulation results with a previous work [6], it is found that the proposed control system has a better performance to increase the intracellular PHB concentration though the maximum PHB content is in the same range. The comparison demonstrates the benefit of the updated setpoints that applied the optimization problems with an effective controller in each time step.

3.2 *Regulatory performance*

For the regulatory performance test, the controller is performed by decreasing the maximum specific growth rate by 10% as a step disturbance in the PHB fermentation process after 20 h.

The process conditions are the same as the closed-loop system in previous section. The results of the regulatory performance test can be shown in Figure 5. This test is proposed as the change of growth rate in the process is a problem that generally occur during the operation by using the mixed culture. The results show that proposed controller has ability to handle the disturbance by adjusting the feed flow rate of the carbon and nitrogen sources according to the change of the cell growth. Note that this work focused on the development of a control system for a fed-batch reactor, and then investigate the process responses by the simulation. The model system was validated by the implementation of the fed-batch reactor in the previous works [11], [13], [14]. Further investigation of the developed control scheme can be conducted by implementing the developed control algorithm (i.e. the flow diagram of Figure 3) to the industrial-scale process.

The integral action in the control structure of this work is used to compensate the process/model mismatch to improve robustness of the fed-batch reactor through the nutrient feeding strategies during the process operation.

4 Conclusions

The enhancement of intracellular PHB accumulation in the fed-batch bioreactor by applying the I/O linearization control technique was proposed in this work. The proposed control strategy composed of an optimizer for calculation of the optimal setpoints which applies C/N ratio that maximize the PHB accumulation through

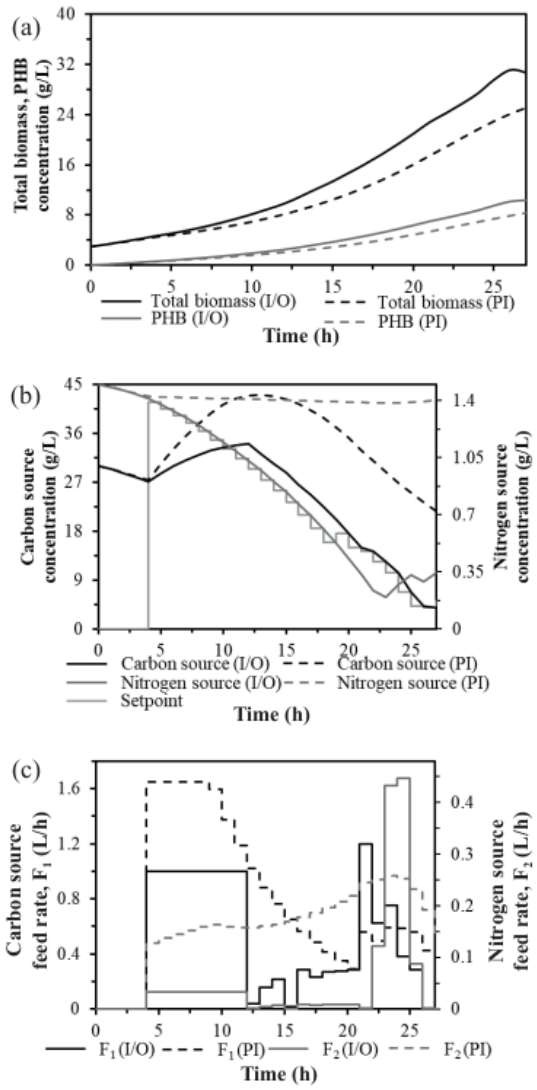

Figure 5: Process response under the regulatory test (a) total biomass and intracellular PHB accumulation (b) carbon and nitrogen source concentration (c) nutrients feed rate.

the carbon and nitrogen source feed flow rate. The control performance of the proposed control system is investigated using a discrete-time approach and compare the process responses with a PI controller that applied the updated setpoints. The simulation results showed that the proposed control strategy has ability to enhance the intracellular PHB accumulation with more optimal feed rate of the carbon and nitrogen sources compared to the modified PI controller. Control performance tests indicate that the proposed control strategy successfully regulates the process according to desired targets and handles the process disturbance effectively.

Acknowledgments

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