Research Article

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Optimization-based control strategy for a large-scale polyhydroxyalkanoates production in a fed-batch bioreactor using a coupled PDE–ODE system

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Abstract: Control strategy development for fed-batch bioreactor (FBBR) plays an important role in the improvement of polyhydroxyalkanoate (PHA) production. To develop a feeding strategy for PHA production in a large-scale FBBR, an optimization-based control scheme that considers nutrient dispersion is proposed in this work. A coupled partial differential equations and ordinary differential equation model is proposed to describe the axial-dispersed nutrient and well-dispersed microbial dynamics with process constraints. An analytical model predictive control (AMPC) method that applies integrated variables of nutrients is employed to develop the real-time control system. The control objective is to regulate the PHA concentration at the updated set points by adjusting the nutrient feed rates; a process disturbance is introduced to evaluate the control robustness. Simulation experiments of a fed-batch operation are conducted to investigate the performance of the developed controller; the controlled output is designed to track the updated set points corresponding to the biomass concentration. Results of closed-loop and regulatory systems showed that the proposed control strategy could provide more productivity (33–38%) compared to the applied PI controller. The performance test demonstrates that the developed control system could apply the biomass concentration for updating set points, provide the optimal control actions that promote PHB accumulation and handle the disturbance effectively.

Keywords: polyhydroxyalkanoates production, fed-batch bioreactor, coupled PDE–ODE model, analytical model predictive control, nonlinear optimization-based control

Nomenclature

A, B	constant matrices
D	differential operator
F_N	feed rate of nitrogen source ($L{\cdot}h^{-1}$)
F_S	feed rate of substrate ($L{\cdot}h^{-1}$)
h	nonlinear functions
H	medium height (cm)
k_1, k_2, k_3, k_4, k_5	kinetic constants
K_{sr}	saturation constant
N	nitrogen concentration ($g{\cdot}L^{-1}$)
N_f	inlet concentration of nitrogen source ($g{\cdot}L^{-1}$)
n_k	dimensionless exponent
Pe	Peclet number
S	substrate concentration ($g{\cdot}L^{-1}$)
S_f	inlet concentration of substrate ($g{\cdot}L^{-1}$)
S_m	carbon/nitrogen ratio for $\mu = 0$
t	time (h)
u	vector of manipulated input
V	working volume (L)
W_N, W_C	weighting factors
X	total biomass concentration ($g{\cdot}L^{-1}$)
y	output variables
y_{sp}	output set points
z	spatial coordinates

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Greek letters

ε	tuning parameter
η	error dynamic parameter
λ	tuning parameter
μ_m	maximum growth rate (h^{-1})
$v^o(k)$	compensated output set point
ξ^p	state variables depending on spatial coordinate and time
ξ^o	state variables depending time
$\tilde{\xi}^p$	lumped variable of PDE subsystem
τ	mean residence time (h)

Subscript

lb	lower bound
sp	set point
ub	upper bound

Superscript

–	measured variable
~	lumped variable
^	estimated state

1 Introduction

Polyhydroxyalkanoates (PHAs) are intracellular-storage biopolymers synthesized by a wide range of microorganisms, which present biodegradable thermoplastic properties. The biopolymers in the family of PHAs, such as polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV), present many beneficial properties for such applications as medical uses and food packaging [1–4]. Although biodegradable polymers have been considered as a potential solution to handle the plastic waste problem, the large-scale production of biopolymers is limited because of the high production cost – which is about 4–10 times higher than that of petrochemical-based polymers [5]. PHAs are energy-storage polymers of microbial cells; PHA accumulation can be promoted under unfavorable growth conditions [6,7]. Since cell growth and polymer accumulation involve influencing factors, the process design of optimal operation for the effective production of PHAs is one of the major challenges.

The process operation of fed-batch fermentation is determined by the mechanism of PHA synthesis. There are two main stages for PHA production: microbial growth with adequate nutrients and PHA accumulation with nutrient limitation [8,9]. Because cell growth and polymer production could be inhibited by a high substrate concentration, fed-batch fermentation has been applied to improve PHA productivity [10]. Literature works have proposed prescribed feeding policies to enhance PHA production in a fed-batch bioreactor (FBBR) [11–13]. The feeding rates of limiting nutrients were optimized and implemented to improve the PHB productivity in fed-batch cultivation [11]. This work indicated that model-based optimization could be particularly suitable for the provision of complicated limiting nutrient conditions for PHB production. Khanna and Srivastava developed a mathematical model to optimize an FBBR; two different nutrient-feeding strategies are selected offline and are conducted for a fed-batch operation [12]. The experimental results showed that the fed-batch operation could increase the PHB productivity by 2.6 times compared to the batch. The developed policies were based on experimental implementations; the obtained results demonstrated a considerable increase in PHA productivity with regard to other previous works. To contemplate the uncertain kinetics and unknown perturbations that are inevitable in online operations, process models have been developed and applied in model-based fed-batch cultivation [14–16]. However, the mathematical models developed by assuming fully dispersed broth in FBBR may be absent in the effect of dispersion in large-scale production. A dispersion model of a large fed-batch bioreactor was proposed for PHB production by *Ralstonia eutropha* [17]. In this study, experiments were conducted to validate the developed kinetic model, and the results showed the potential for improving the PHB production in large-scale bioreactors by considering the dispersion effect.

During the last decades, some control strategies have been proposed to improve the control performance and closed-loop robustness of FBBR for PHA production [17–21]. A dynamic optimization technique was applied to develop optimal feed rate profiles of carbon and nitrogen sources that can improve PHB productivity in a fed-batch bioreactor [18]. The adaptive control schemes that applied the constraint discrete-time optimal control algorithm were developed for PHB production [19]. Recently, there have been control strategies proposed based on input/output (I/O) linearization and real-time optimization to develop advanced feeding strategies for fed-batch fermentation [21,22]. Srisuddee et al. proposed a control method based on the I/O linearization technique for a fed-batch bioreactor described by an ODE model [20]. Ochoa et al. applied a soft-sensor prediction block based on previous work to develop the real-time optimization and control for a fed-batch production of PHAs in a large-scale bioreactor [21,22].

However, in large-scale production, the feed substrate and nitrogen source with high concentrations in a fed-batch bioreactor are placed on the upper surface of the broth while the microbial cells are well dispersed in the agitated broth. During the process operation, the changing of substrate and nitrogen concentration in the broth affects the carbon-to-nitrogen (C/N) ratio that could promote the cell and polymer productions. Though some studies have shown the effects of nutrient dispersion on the PHA production in large-scale bioreactors, most of the control strategies have been developed based on fully dispersed behavior. Therefore, the performance of traditional controllers that assume full dispersion of nutrients may be limited by the complex interaction between the dispersed nutrients and microbial cells [23–26]. Thus, this work aims to develop an alternative optimization-based control strategy that considers the axial dispersion of the substrate and nitrogen source coupled with the well dispersion of biomass. The control objective is to control the PHA concentration at the updated set points by adjusting the substrate and nitrogen feed rates; a process disturbance is introduced to evaluate the control robustness. To investigate closed-loop responses, the dynamics of substrate and nitrogen-source concentrations are considered as distributed variables and can be described by PDEs, while that of the well-dispersed biomass and PHA concentrations are modeled by ODEs. The control strategy is based on the analytical model predictive control (AMPC) method that applies a coupled PDEs–ODEs model for a fed-batch bioreactor. The AMPC method has presented the advantage of optimal manipulation to prevent an aggressive control action for the continuous reactor system described by PDEs–ODEs model in some previous studies [27]. For the fed-batch reactor, the optimal control action could promote polymer production and avoid the effect of substrate inhibition that reduces microbial growth simultaneously. A large-scale fed-batch bioreactor for PHB production is considered a case study in this work. The developed AMPC-based controller is combined with a nonlinear state estimator and integrator to estimate the unmeasured variables and handle process disturbances. The main contributions of this work, beyond the novelty of the problem formulation, lie in integrating the dispersion effects of concentrated substrates of the controller, compensating unknown perturbations that are inevitable in the real-time operation, and providing control actions that optimize the nutrient feed rates to enhance the biomass and PHA production.

2 Materials and methods

2.1 Problem formulation

Consider the system of a mathematical model for a fed-batch bioreactor used for large-scale polyhydroxyalkanoates production in Eq. 1. Dynamics of the state variables are described by a coupled parabolic partial differential equations and ordinary differential equation (PDE–ODE) system as follows:

$$\begin{aligned}\frac{\partial \xi^p(z,t)}{\partial t} &= A\frac{\partial^2 \xi^p(z,t)}{\partial z^2} + B\frac{\partial \xi^p(z,t)}{\partial z}\\ &\quad + \mathbb{F}_p(\xi^p(z,t),\xi^o(t))\frac{\partial \xi^o(t)}{\partial t}\\ &= \mathbb{F}_o(\xi^o(t),\tilde{\xi}^p(t),u(t))y\\ &= h(\xi^p(z,t),\xi^o(t))|_{z=H}\end{aligned} \tag{1}$$

where

$$\tilde{\xi}^p(t) = \int_0^H \xi^p(z,t)\mathrm{d}z$$

with the following initial and boundary conditions:

$$\xi^p(z,0) = \xi_0^p,\ \xi^o(0) = \xi_0^o$$

$$z = 0:\ \frac{\partial \xi^p(z,t)}{\partial z} = 0$$

$$z = H:\ \frac{\partial \xi^p(z,t)}{\partial z} = 0$$

where $\xi^p(z,t)$ denotes the vector of states depending on the spatial coordinate (axial liquid level) and time, $\xi^o(t)$ denotes the vector of time-dependent state variables, $\tilde{\xi}^p(t)$ denotes the integrated state of ξ^p, $u(t)$ denotes the vector of manipulated variables, y denotes the vectors of the output variables, $\mathbb{F}_p$, $\mathbb{F}_o$ and h are nonlinear functions, $z \in [0,H]$ is the spatial coordinate, $t \in [0,t_f]$ is the time, and A and B are matrices.

2.2 Control system design

Considering the polymer production in the fed-batch bioreactor, the agitated cells with intracellular PHAs consume the dispersed substrate and nitrogen source in the broth. To illustrate the effects of nutrient dispersion in the fed-batch bioreactor, an open-loop simulation of PHA production is conducted. A set of constant nutrient feed rates is applied; the substrate and nitrogen concentrations are considered distributed variables. The open-loop profile of substrate concentration along the axial distance (dispersed broths) for 5, 10, 15, and 20 h is shown in Figure 1. More details of the mathematical model and operating conditions are given in the next sections.

The concentrated substrate is fed to the bioreactor and is consumed by the microbial cells. The figure illustrates that a large-scale fed-batch operation intensifies the lack of homogeneity of the broths; the agitated cells at different positions in the bioreactor are affected by nutrient dispersion [17]. For the coupled PDE–ODE system, the dynamic behaviors of the states ξ^p and ξ^o are bidirectional interconnection. Consider a compact form of the system in Eq. 1:

$$\begin{aligned} \dot{\xi}^o &= \mathbb{F}_o(\xi^o, \tilde{\xi}^p, u) \\ y^o &= \xi^o \end{aligned} \tag{2}$$

where u is the vector of the manipulated inputs. An analytical model predictive control strategy based on I/O linearization, which has been successfully applied to continuous reactor systems, is modified to formulate the optimization problem for the fed-batch bioreactor in this work [27–30]. The nonlinear optimization is established to provide the optimal control actions by minimizing the squared error between the existing state and the updated target in each time step. The constraint optimization problem can be formulated as the following equation; then, it is solved for the manipulated input at each time step ($t \in [t_0, t_f]$):

$$\min_u \left[\frac{(\varepsilon D + 1)^r y^o - v^o}{\varepsilon} \right]^2 \tag{3}$$

subject to

$$\begin{aligned} &\dot{\xi}^o = \mathbb{F}_o(\xi^o, \tilde{\xi}^p, u) \\ &u_{\mathrm{lb}} \le u \le u_{\mathrm{ub}} \\ &v^o_{\mathrm{lb}} \le v^o \le v^o_{\mathrm{ub}} \end{aligned}$$

where D is the differential operator (i.e., $D = \frac{\mathrm{d}}{\mathrm{d}t}$), ε is the tuning parameter that is constant and adjustable. y^o is the vector of outputs, u_{lb} and u_{ub} denote the lower bound and upper bound of the manipulated input u, respectively, v^o is the reference output setpoint obtained from the real-time calculation at the current time. The relative order (r) of the controlled outputs y^o with respect to manipulated input u of the dynamic behaviors of the states ξ^o is investigated for the smallest integer that provides $\partial[\mathrm{d}^r y^o/\mathrm{d}t^r]/\partial u \neq 0$ [27]. To design the optimization-based control scheme for the system in Eq. 2, the differential operator is applied to the controlled output y^o, of which a relative order number is equal to 1. Then, the optimization problem in Eq. 3 can be rewritten as the following equation:

$$\min_u \left[\frac{\varepsilon \mathbb{F}_o(\xi^o, \tilde{\xi}^p, u) + y^o - v^o}{\varepsilon} \right]^2 \tag{4}$$

subject to:

$$\begin{aligned} &\dot{\xi}^o = \mathbb{F}_o(\xi^o, \tilde{\xi}^p, u) \\ &u_{\mathrm{lb}} \le u \le u_{\mathrm{ub}} \\ &v^o_{\mathrm{lb}} \le v^o \le v^o_{\mathrm{ub}} \end{aligned}$$

The solutions from the optimization problem are obtained by applying the distributed process model, and the cell growth and polymer accumulation can be employed to provide the optimal control actions for the fed-batch bioreactor.

2.3 Mathematical model of a fed-batch bioreactor for PHB production

Among the family of PHAs, PHB is an important biodegradable polymer that presents beneficial properties and can be synthesized by various microorganisms. With the mechanical properties (e.g., tensile strength, flexural modulus)

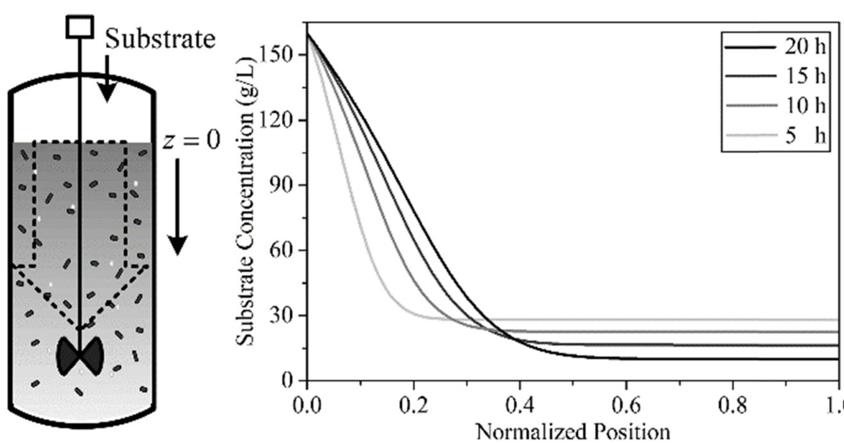

Figure 1: Spatial dispersion of substrate concentration.

that are similar to polypropylene, the PHB market is predicted to reach $98 million by 2024 [31]. In this work, a fed-batch bioreactor of PHB production is considered a case study (see Figure 2 for a schematic diagram of the fed-batch bioreactor).

In this example, the fermentation is operated in the fed-batch bioreactor for 30 h. The prepared inoculum (*Cupriavidus necator*) is supplied into a 500 L bioreactor. The culture medium is composed of 75 vol% of sugarcane vinasses and 25 vol% of molasses [22,32]. The process is initially performed in batch operation for 2 h; microbial cells are well dispersed in the agitated medium. Then, the process is performed in a fed-batch operation, where the substrate (molasses) and nitrogen source are fed to the bioreactor to manipulate the PHB production. In recent years, some research articles have proposed the online monitoring of substrate, biomass, and PHB by using sensors, gas chromatography, infrared spectroscopy, or estimated by a reliable measurement [19,21,33,34]. Thus, the substrate, biomass and PHB concentrations, and working volume are assumed to be measurable in real-time during the operation. However, the nitrogen concentration cannot be measured online [22]; therefore, a nonlinear state estimation is applied in this work. A nonlinear first-principles model for the fed-batch bioreactor is developed, with additional simplified assumptions considered in the one-dimensional PHB bioreactor model:

1. The biomass and PHB concentrations in the bioreactor are assumed to a follow fully dispersed behavior.
2. The dispersion of substrate and nitrogen sources is considered to be one-dimensional.
3. The microorganisms consume substrate and nitrogen sources to produce biomass and PHB. By-product production is neglected.

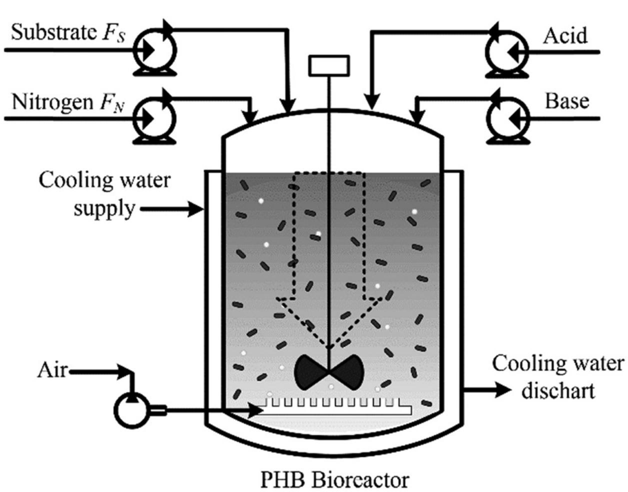

Figure 2: Schematic diagram of a typically PHB fed-batch bioreactor.

4. The temperature, pH, and dissolved oxygen of the bioreactor are effectively regulated in a range [22]; the impact of these factors on microbial growth can be ignored.
5. The substrate concentration, total biomass concentration, PHB concentration, and working volume are measurable in real time during the operation.

The governing equations of the fed-batch bioreactor for PHB production are composed of dynamic behaviors of the substrate concentration, nitrogen concentration, biomass concentration, PHB concentration, and working volume.

2.3.1 Mass balance of the distributed variables

The process model for fed-batch fermentation was reformulated in previous work to include dispersion terms of nutrients with Pe = 20 [25,26]. The previous studies showed a significant effect of axial dispersion on the kinetics of PHB formation; more details and equations are available in the literature. The conservation of substrate and nitrogen sources is distributed along the liquid level of the fed-batch bioreactor. The dynamics of substrate and nitrogen concentrations can be described as follows:

$$\begin{aligned}\tau\frac{\partial S(z,t)}{\partial t} &= \frac{1}{\mathrm{Pe}}\frac{\partial^2 S(z,t)}{\partial z^2} - \frac{\partial S(z,t)}{\partial z}\\ &\quad - \tau\left[\frac{S_f F_S}{V(t)} - k_1\frac{\mathrm{d}X(t)}{\mathrm{d}t} - k_2 X(t)\right.\\ &\quad \left. - \frac{S(z,t)}{V}\frac{\mathrm{d}V(t)}{\mathrm{d}t}\right] \\ \tau\frac{\partial N(z,t)}{\partial t} &= \frac{1}{\mathrm{Pe}}\frac{\partial^2 N(z,t)}{\partial z^2} - \frac{\partial N(z,t)}{\partial z}\\ &\quad - \tau\left[\frac{N_f F_N}{V(t)} - k_3\frac{\mathrm{d}X(t)}{\mathrm{d}t} - \frac{N(z,t)}{V}\frac{\mathrm{d}V(t)}{\mathrm{d}t}\right]\end{aligned} \tag{5}$$

with the boundary and initial conditions:

$$z = 0 : \frac{\partial S(z,t)}{\partial z} = 0 \qquad \frac{\partial N(z,t)}{\partial z} = 0$$

$$z = H : \frac{\partial S(z,t)}{\partial z} = 0 \qquad \frac{\partial N(z,t)}{\partial z} = 0$$

$$t = 0 : S(z,0) = S_0 \qquad N(z,0) = N_0$$

where $S(z, t)$ denotes substrate concentration, $N(z, t)$ denotes nitrogen concentration, $X(t)$ denotes total biomass concentration, $V(t)$ denotes the working volume,

τ denotes the mean residence time, $z \in [0, H]$ denotes spatial distance (dispersed broths), Pe denotes the Peclet number, k_1, k_2, and k_3 denote kinetic constants, S_f and N_f denote the inlet concentration of substrate and nitrogen source, and F_S and F_N denote the feed rate of substrate and nitrogen source, respectively.

2.3.2 Mass balance of the lumped variables

In general, the process is initially operated in batch operation, and the inoculated cells are well dispersed in the agitated medium. After the process is performed in fed-batch operation, concentrated substrate and nitrogen source are fed to the bioreactor, which results in a complex interaction between the dispersed nutrients and microorganisms. The dynamics of the total biomass concentration, accumulated PHB concentration, and working volume can be written as follows:

$$\begin{aligned} \frac{\mathrm{d}X(t)}{\mathrm{d}t} &= \left[\mu_m\left(\frac{\frac{\tilde{N}(t)}{\tilde{S}(t)}}{\frac{\tilde{N}(t)}{\tilde{S}(t)} + K_{\mathrm{sr}}}\right)\left(1-\left(\frac{\frac{\tilde{N}(t)}{\tilde{S}(t)}}{S_m}\right)^{n_k}\right)\right]X(t) \\ &\quad - \frac{X(t)}{V(t)}\frac{\mathrm{d}V(t)}{\mathrm{d}t} \\ \frac{\mathrm{d}P(t)}{\mathrm{d}t} &= k_4\frac{\mathrm{d}X(t)}{\mathrm{d}t} + k_5X(t) - \frac{P(t)}{V(t)}\frac{\mathrm{d}V(t)}{\mathrm{d}t} \\ \frac{\mathrm{d}V(t)}{\mathrm{d}t} &= F_S(t) + F_N(t) \end{aligned} \tag{6}$$

where

$$\tilde{S}(t) = \int_0^H S(z, t)\mathrm{d}z \text{ and } \tilde{N}(t) = \int_0^H N(z, t)\mathrm{d}z$$

with the initial conditions:

$$\begin{aligned} t = 0 : X(0) &= X_0 \\ P(0) &= P_0 \\ V(0) &= V_0 \end{aligned}$$

where μ_m denotes the maximum growth rate, $\tilde{S}(t)$ and $\tilde{N}(t)$ are integrated states of substrate and nitrogen-source concentration, K_{sr} denotes saturation constant, S_m denotes carbon/nitrogen ratio for $\mu = 0$, n_k denotes dimensionless exponent, and k_4 and k_5 are kinetic constants. The process parameter values are presented in Table 1 [22,26].

2.4 Formulation of the control system

Since the growth and PHB accumulation of microbial cells depending on the dispersed carbon and nitrogen source, the control objective of this example process is to regulate the PHB concentration (P) at the updated set points by manipulating the feed rates of the nitrogen source (F_N) and the substrate (F_S). The application of process control requires the online state estimator for unmeasurable states. In this work, the integrated state of nitrogen concentration ($\tilde{N}$) can be estimated by applying a dynamic simulation of a finite-element model. More details of the state estimation are given in the next subsection. Because the cell growth can be inhibited by a high carbon-to-nitrogen (C/N) ratio, an optimum C/N ratio is defined for the fed-batch operation [16,35]. The process models of Eqs. 5 and 6 are applied to the optimization problem of Eq. 4, and the constrained optimization problem for the manipulated input ($u = F_N$) is solved at each time instant:

Table 1: Parameters of the fed-batch bioreactor for PHB production

Symbol	Parameter	Value
μ_m	Axial dispersion coefficient	0.437 h^{-1}
S_m	Carbon/nitrogen ratio for $\mu = 0$	0.3 g N/g S
K_{sr}	Saturation constant	0.0697
n_k	Dimensionless exponent	1.490
Pe	Peclet number	20
k_1	Kinetic constant	1.831
k_2	Kinetic constant	0.067
k_3	Kinetic constant	0.6511
k_4	Kinetic constant	0.300
k_5	Kinetic constant	0.008

$$\min_u J[\bar{P}, \bar{X}, \tilde{N}, \bar{S}, \bar{V}, F_S, u, y_{\mathrm{sp}}] \tag{7}$$

$$J = \frac{w_N}{\varepsilon^2}\left[\left(k_4\left(\mu\bar{X} - \frac{\bar{X}}{\bar{V}}(F_S + F_N)\right) + k_5\bar{X} - \frac{\bar{P}}{\bar{V}}(F_S + F_N)\right) + \bar{P} - y_{\mathrm{sp}}\right]^2$$

where

$$\mu = \mu_m\left(\frac{\frac{\tilde{N}}{\bar{S}}}{\frac{\tilde{N}}{\bar{S}} + K_{\mathrm{sr}}}\right)\left(1-\left(\frac{\frac{\tilde{N}}{\bar{S}}}{S_m}\right)^{n_k}\right)$$

$$F_S = w_C\left(\frac{N_f u}{S_f}\right) \text{ and } y_{\mathrm{sp}} = P_d\bar{X}$$

$$w_C = \begin{pmatrix} w_{C,1}, \bar{S}/\tilde{N} \le R_{CN} \\ w_{C,2}, \bar{S}/\tilde{N} > R_{CN} \end{pmatrix}$$

subject to:

$$\begin{aligned} &\dot{X} = \varphi_1(\tilde{N}, \bar{S}, X, P, V) \\ &\dot{P} = \varphi_2(X, P, V) \\ &u_{\mathrm{lb}} \le u \le u_{\mathrm{ub}} \\ &y_{\mathrm{sp,lb}} \le y_{\mathrm{sp}} \le y_{\mathrm{sp,ub}} \end{aligned}$$

where $\bar{S}$, $\bar{P}$, and $\bar{X}$ are the measured states of substrate concentration, PHB concentration, and total biomass concentration, respectively. $\tilde{N}$ is the integrated state of nitrogen concentration, $\bar{V}$ is the current volume, F_N is the manipulated input (u), y_{sp} is the set point, w_N and w_C are weighting factors, R_{CN} and P_d are the defined carbon/nitrogen (C/N) ratio and PHB productivity, respectively, and ε is tuning parameter. Note that the defined C/N ratio and PHB productivity are based on the experimental results in previous works [17,22,35]. The set point calculator applies the defined PHB productivity to create the set point trajectory that relies on the current biomass concentration.

2.5 The control approach

For the control application, real-time state estimation for the unmeasurable variables is required for providing the state feedback variables. In this work, the unmeasurable state and the state derivatives are estimated by applying a finite-based, nonlinear state estimator, and the process model of Eqs. 5 and 6. A schematic diagram of the proposed control structure is shown in Figure 3.

The experimental simulation of the example process is conducted by computing software such as MATLAB and COMSOL Multiphysics. A compact equation of the nonlinear state estimator can be written as follows:

$$\dot{\hat{\xi}}^p = \hat{\mathbb{F}}_p(\hat{\xi}^p, \hat{\xi}^o)\dot{\hat{\xi}}^o = \hat{\mathbb{F}}_o(\hat{\xi}^p, \hat{\xi}^o, u)\hat{y} = \hat{h}(\hat{\xi}^p, \hat{\xi}^o) \qquad (8)$$

where the vectors of estimated states are $\hat{\xi}^p = [\hat{S}\hat{N}]^T$, $\hat{\xi}^o = [\hat{X}\hat{P}]^T$, and $\hat{y} = [\hat{P}]$. The nonlinear state estimator in Eq. 8 requires the manipulated input u from the AMPC controller to calculate the estimated state $\hat{N}$ at each time instant. Additionally, first-order integral action is combined to compensate for unmeasured disturbances as presented in the following equations [36]:

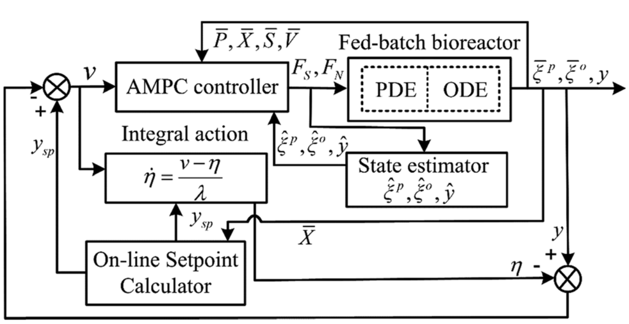

Figure 3: Schematic diagram of the developed control system for the fed-batch bioreactor.

$$\dot{\eta} = \frac{v - \eta}{\lambda}$$
$$v = y_{sp} - \eta \qquad (9)$$

where η is the integral of error of the controlled output y. λ is a positive constant, and v is the compensated set point. To estimate the unmeasured state, state derivatives and ensure the closed-loop stability of the developed control system, the integrator and finite-element-based state estimator are combined with the AMPC controller. The optimization problem for the controller can be written in a function of variables as follows:

$$\min_u J[\bar{P}, \bar{X}, \hat{N}, \bar{S}, \bar{V}, F_S, u, y_{sp}] \qquad (10)$$

subject to:

$$\dot{X} = \varphi_1(\tilde{N}, \tilde{S}, X, P, V)$$
$$\dot{P} = \varphi_2(X, P, V)$$
$$u_{lb} \le u \le u_{ub}$$
$$y_{sp,lb} \le y_{sp} \le y_{sp,ub}$$

Implementation of the developed control system requires feedback measurements from the available sensors and state estimator. To avoid a high substrate concentration caused by substrate accumulation, the input constraints of the feed streams need to be specified.

3 Results and discussion

The process behaviors of the fed-batch bioreactor are simulated by applying the coupled PDE–ODE system in Eqs. 5 and 6. In the simulation, the initial conditions are applied: $S(z, 0) = 32\,\mathrm{g{\cdot}L^{-1}}$, $N(z, 0) = 2.1\,\mathrm{g{\cdot}L^{-1}}$, $X(0) = 8.75\,\mathrm{g{\cdot}L^{-1}}$, $P(0) = 1\,\mathrm{g{\cdot}L^{-1}}$, and $V(0) = 280\,\mathrm{L}$. For the feedstock, molasses is used as the feed substrate with a sugar concentration (S_f) of $160\,\mathrm{g{\cdot}L^{-1}}$ while the feed nitrogen concentration (N_f) is $28\,\mathrm{g{\cdot}L^{-1}}$. The temperature, pH, and dissolved oxygen of the fed-batch bioreactor are controlled at 30°C, 7, and 40%, respectively [22]. The PHB concentration is controlled by manipulating the nitrogen and substrate feed rates of the closed-loop system. A traditional PI controller is applied to compare the control performance. The PI controller employs the updated set point, and the internal model control (IMC) tuning method is applied to tune the PI controller. In the closed-loop simulations, the process parameters are applied: $\varepsilon = 20$, $u_{lb} = 0.01\,\mathrm{L{\cdot}h^{-1}}$, $u_{ub} = 10\,\mathrm{L{\cdot}h^{-1}}$, $P_d = 0.4$, $w_N = 0.7$, $w_{C,1} = 7$, $w_{C,2} = 0.1$, $R_{CN} = 20$, $\lambda = 0.001$, $y_{sp,ub} = 3\,\mathrm{g{\cdot}L^{-1}}$, and $y_{sp,ub} = 12\,\mathrm{g{\cdot}L^{-1}}$ [17,22,37]. The PI controller is simulated with a set of tuning parameters, $K_p = -0.3$

and $K_I = -0.003$. The closed-loop response of the PHB concentration, response of the substrate and nitrogen concentration, response of biomass concentration, the profile of the substrate, and nitrogen feed rate are shown in Figures 4–7, respectively.

At the beginning of the fed-batch operation, the proposed controller increases the feed rates of substrate and nitrogen sources to support the growth of microorganisms. An increase in microbial growth, itself resulting from the adequate substrate and nitrogen source, increases the cells for polymer accumulation, which increases the PHB concentration [8]. Then, the control actions are optimally adjusted to minimize the error between the response of the PHB concentration and the updated set points. As shown in Figure 4, the PHB accumulation increases rapidly after the bioreactor is regulated with a low-nitrogen supply condition.

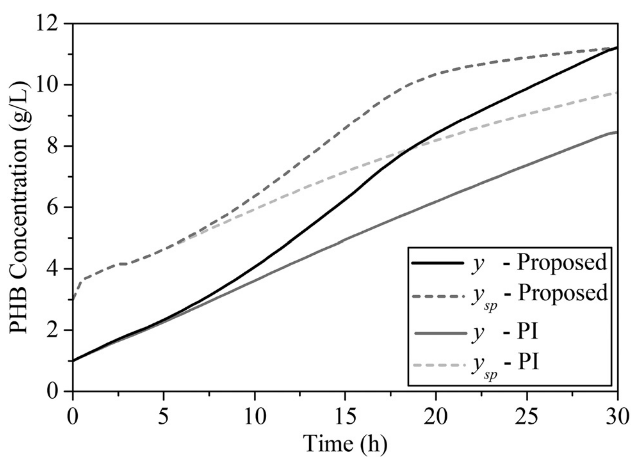

Figure 4: PHB concentration response of the closed-loop system.

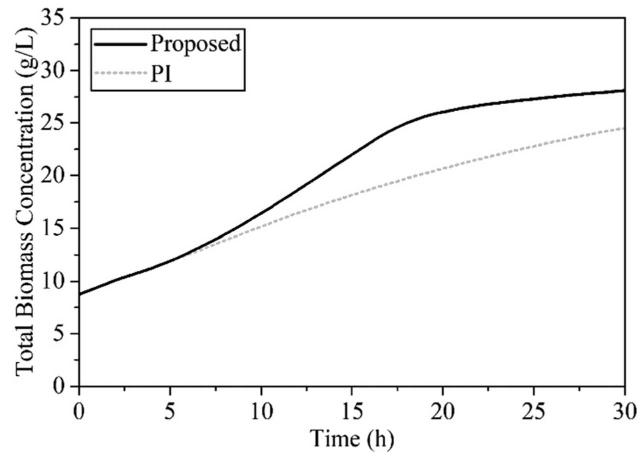

Figure 6: Biomass concentration profile corresponding to the closed-loop system of Figure 4.

The lack of a nitrogen source decreases the intracellular enzyme for cell growth, and the substrate is consumed for the enhancement of PHB production [9]. The different results corresponding to cultivations carried out with the various types of cultures and substrates are reported in previous research works. For the experimental studies reporting PHA production by *C. necator* from a vinasses–molasses mixture, cultivation in lab- and pilot-scale bioreactors have been performed in some works. Cultivations were carried out in a 5-L bioreactor using vinasse with nitrogen and mineral salts [38]. The PHB content (26% of total biomass) was reported as the best value when compared to the application of pure vinasse and vinasse with nitrogen. García et al. reported a value of productivity for PHA production ($0.27\ g{\cdot}L^{-1}{\cdot}h^{-1}$) from fermentation in a 5-L bioreactor using vinasses–molasses mixture [21]. An extended

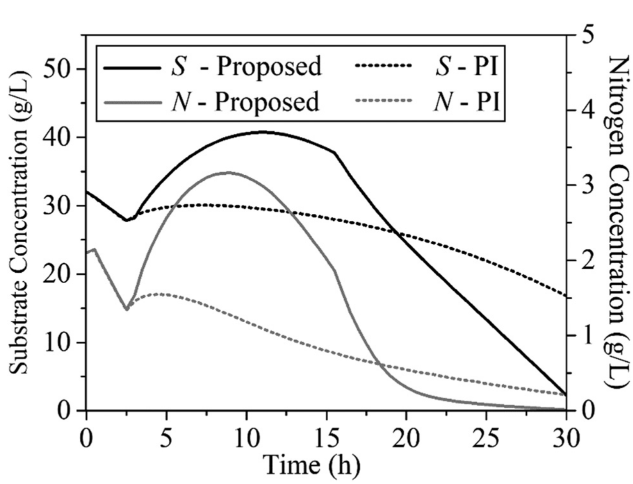

Figure 5: Substrate and nitrogen concentration profiles corresponding to the closed-loop system of Figure 4.

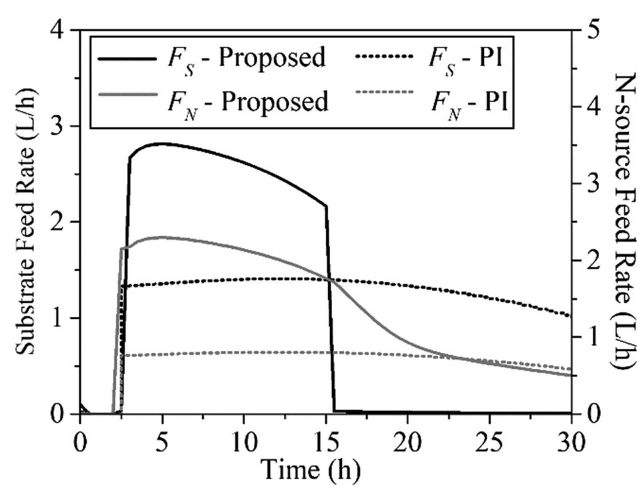

Figure 7: Profile of substrate and nitrogen-source feed rates corresponding to the closed-loop system of Figure 4.

study was conducted in a 500-L bioreactor using similar substrates [22]. The feeding policy for fed-batch operation is developed based on a lumped model; a soft sensor is applied for substrate prediction. Among their developed control strategies, the best value reported for productivity ($0.364\ g{\cdot}L^{-1}{\cdot}h^{-1}$) was obtained in the fermentation that applied the productivity and profitability-oriented optimization. To compare the control performance of the proposed system with similar substrates, cultures, and conditions in this work, the modified PI controller is applied with the updated set points [39,40]. The PHB concentration, content of PHB (% of total biomass), substrate concentration, and nitrogen concentration are shown in Figures 8–11, respectively.

As shown in the figures, the proposed control strategy achieved higher performance than the applied PI controller. The PHB concentrations at the end of the operation obtained from the proposed controller and modified PI controller are 11.27 and $8.45\ g{\cdot}L^{-1}$, while the PHB contents (% of total biomass) are 40.12% and 34.70%, respectively. As explained above, the changing of substrate and nitrogen concentration in the broth during the operation could selectively promote cell and polymer productions. The proposed controller applied the integrated variables of substrate and nitrogen concentrations to update the set points and provide the optimal control actions to promote PHB accumulation. Additionally, the low final substrate and nitrogen-source concentrations shown in Figures 10 and 11 demonstrate that the proposed feeding strategy has the ability to utilize the nutrients effectively. The developed control strategy is evaluated for a regulatory problem to investigate the control robustness. The regulatory test is conducted by using the same initial conditions as the open-loop simulation. An unmeasured step disturbance is introduced by decreasing the feed nitrogen concentration by 10% after 12 h [37]. The responses of the

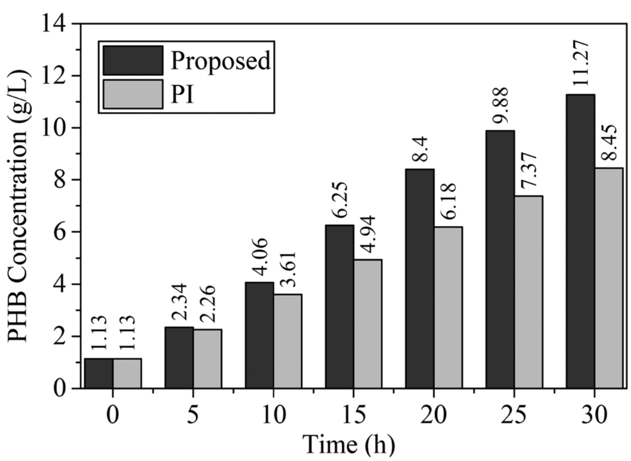

Figure 8: PHB concentration of the closed-loop system.

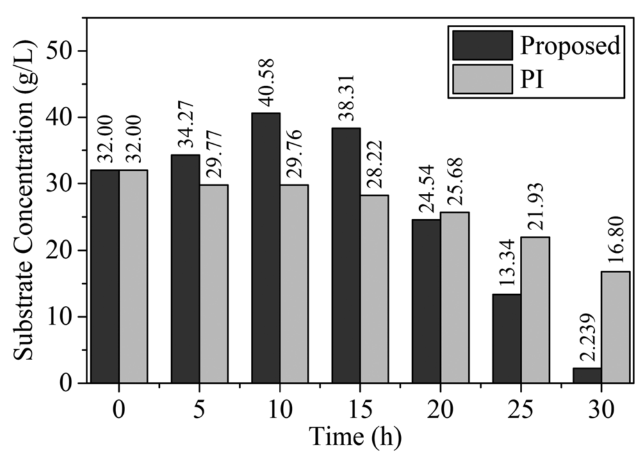

Figure 10: Substrate concentration of the closed-loop system.

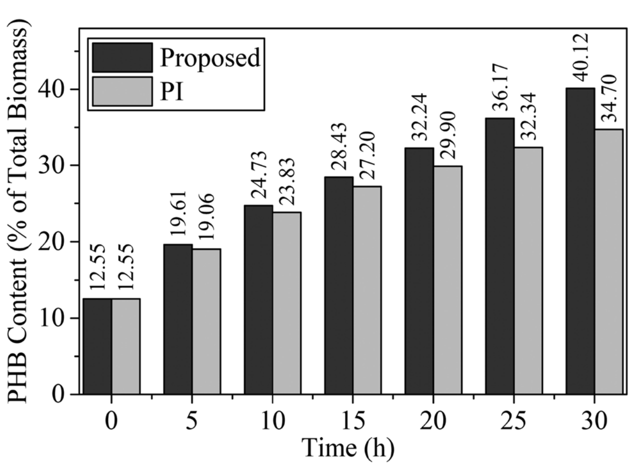

Figure 9: PHB content of total biomass.

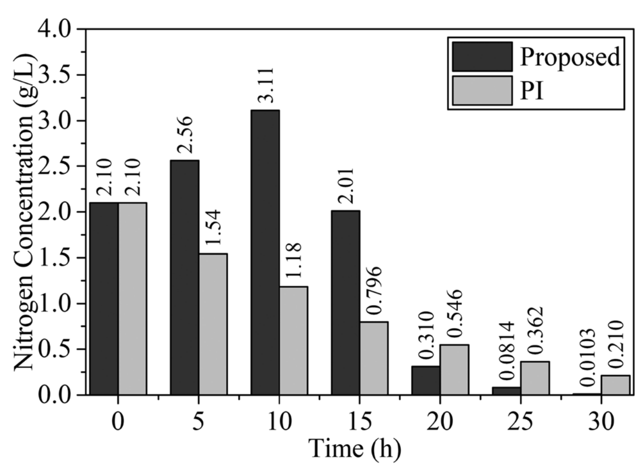

Figure 11: Nitrogen concentration of the closed-loop system.

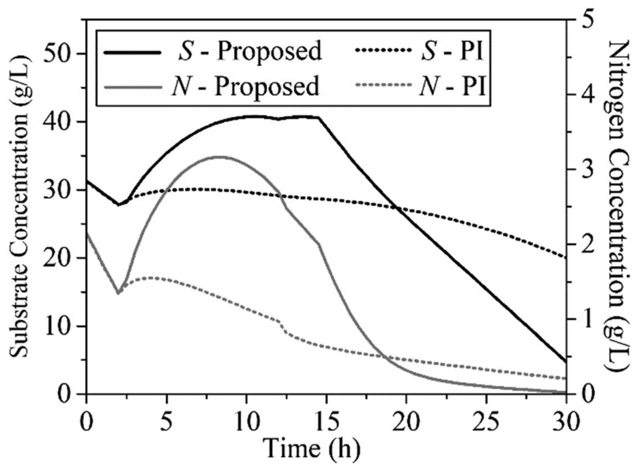

Figure 12: Substrate and nitrogen concentration profiles under performance test.

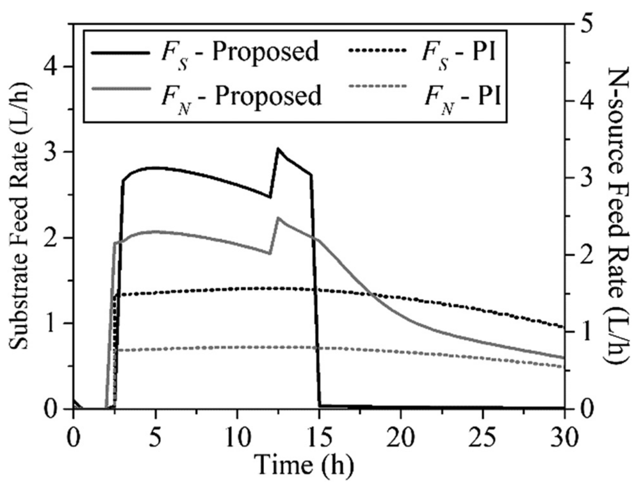

Figure 13: Profile of substrate and nitrogen-source feed rates under performance test.

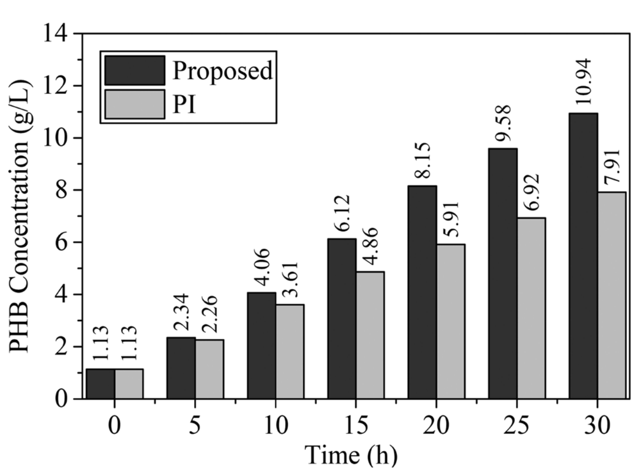

Figure 14: PHB concentration under regulatory test.

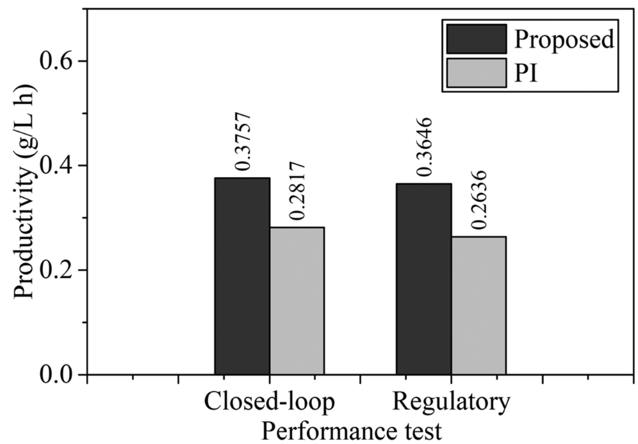

Figure 15: PHB Productivity of the closed-loop and regulatory system.

performance test are shown in Figures 12 and 13, where it can be seen that the developed control scheme successfully rejects the effect of the disturbance and compensates for the reduced feed nitrogen concentration. The PHB concentration and productivity of the performance test are shown in Figures 14 and 15, respectively.

The fed-batch operation with a change in nutrient feed concentration could lead to inconsistency in the polymer production rate (unfortunately, there is no report of the investigation for the PHA production by vinasses/molasses) [37]. Though the disturbance affects PHA production, the proposed strategy updates the trajectory path for set point tracking while the first-order integrator compensates for the error, quickly rejects the disturbance, and improves the process responses. It can be seen from the results that are shown in Figure 15 that the proposed control system could provide more productivity (33–38%) compared to the applied PI controller. From the results, it is clear that the proposed control strategy that considers nutrient dispersion has the ability to enhance PHB production and improve the performance of the fed-batch bioreactor.

4 Conclusions

This article has proposed an optimization-based control strategy to enhance PHA production in the fed-batch bioreactor described by a coupled PDEs–ODEs model. The AMPC-based controller is combined with a nonlinear state estimator and integrator to estimate the unmeasured variables and handle process disturbances; the constrained optimization problem of the controller applies the set point trajectory corresponding to the biomass concentration. In simulation experiments, a change in feed concentration was introduced to investigate control robustness. The results showed that the developed control scheme successfully provides the optimal control actions that enhance the polymer production and follow the updated set point. Control performance of the control strategy under a regulatory test demonstrated the capability to handle the process disturbance and provide adequate control actions efficiently. Additionally, further applications of the control strategy to industrial-scale PHA processes with a greater dispersion effect are expected to obtain better control performance and improve process efficiency.

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