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Fermentation of glycerol using *Clostridium butyricum* for the production of 1,3-Propanediol in a Fed-batch bioreactor using advanced controllers

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Abstract

Due to the continuous growth of soap, detergent, and biodiesel industries, glycerol production as a byproduct has increased substantially, resulting in a significant reduction of its price. Glycerol can be fermented with many bacterial strains to produce several valuable chemicals like 1,3-propanediol (1,3-PD). This organic compound is highly versatile, useful for producing a wide range of polymers such as polyesters, polyester, polyurethane, and trimethylene terephthalate; hence, the industrial production of 1,3-PD is highly appealing. The fed-batch cultivation of *Clostridium butyricum*, using glycerol as substrate, was simulated using Matlab-Simulink and an Orthogonal Collocations on Finite Elements procedure (OCFE) was implemented, using AMPL software, to find the optimal path of substrate concentration in the reactor that could maximize the production of 1,3-PD. Then a Model Predictive Control (MPC) was applied to follow the optimal setpoints, given by AMPL, of substrate concentration and temperature reactor. Finally, the performance achieved in 40 hours of simulation, with a final concentration and productivity of 74.94 g/L and 1.874 g/L/h respectively, gave similar results of 1,3-PD production by other microorganisms like *C. diolis* and *K. pneumoniae*.

Keywords: Fed-batch, *Clostridium butyricum*, Model Predictive Control, Optimum Path, Orthogonal Collocations on Finite Elements, OCFE, AMPL, Matlab Simulink.

1. Introduction

Glycerol is generated as a byproduct of the soap and detergent industries but is also the main byproduct of the biodiesel industry (Kanjilal, 2015; Santibáñez et al., 2011). The biodiesel industry's growth has generated a dramatic surplus of crude glycerol, resulting in a 10-fold decrease in its price in recent years (Santibáñez et al., 2011). Currently, glycerol is used in fermentation processes to obtain 1,3 propanediol (1,3-PD), by means of several bacteria such as *Citrobacter*, *Enterobacter*, and *Clostridium*. In these cases, the bioreactors operate under physiological culture conditions, avoiding the production of harmful residues (Kaur et al., 2012b). 1,3-PD is a biodegradable and versatile organic compound. Its production is of high interest for the textile and thermoplastic industries, as it has a wide range of applications as a monomer for polyesters, polyether, polyurethane, and trimethylene terephthalate (PTT) (Kaur et al., 2012b).

Clostridium butyricum is a suitable microorganism to

produce 1,3-PD from glycerol. This is a strict anaerobic mesophile strain that can metabolize complex substrates and tolerates higher glycerol concentrations than *Clostridium diolis* and *K. pneumoniae* (see Table 1) (Colin et al., 2001). A model of *Clostridium butyricum* fedbatch cultivation was used to optimize the process with the Rosenbrock algorithm (Kaur et al., 2013); a final 1,3-PD concentration of 64 g/L was obtained in 47 h of cultivation. Applying a nonlinear on/off control, a final 1,3-PD concentration of 108 g/L in 39 h of operation has been achieved (Niu et al., 2018). With the microorganism *K.pneumoniae*, a control tuned with particle swarm optimization (PSO) was able to achieve 78 g/L of 1,3-PD in 24 h of cultivation (Liu et al., 2013), while a fermentation process modelled with a nonlinear switched time-delay system yielded 57 g/L of 1,3-PD in 20 h (Liu et al., 2017).

Table 1. Final concentration (q) and volumetric productivity (p) of 1,3-PD reported in literature.

Reference	P [g/L]	Microorganism	q [g/L/h]
Kaur *et al.* (2013)	64	*C. diolis*	1.36
Liu *et al.* (2013)	78	*K. pneumoniae*	3.25
Niu *et al.* (2018)	108	*K. pneumoniae*	2.77
Liu *et al.* (2017)	57	*K. pneumoniae*	2.85
This study	75	*C. butyricum*	1.87

In this work, the optimal control strategy that maximizes the production of 1,3-PD was determined using AMPL in a fedbatch cultivation of *Clostridium butyricum* using crude glycerol as substrate. The controlled variables in the bioreactor were glycerol concentration and reactor temperature using a Model Predictive Control.

2. Materials and Methods

2.1. Control objectives

The main control objective is the maximization of 1,3-PD production. This maximization requires manipulating the glycerol feed rate to control its concentration in the bioreactor. Growth of *Clostridium butyricum* and production of 1,3-PD are sensitive to the culture's glycerol levels. The secondary control objective is to ensure that the culture temperature is optimal for bacterial growth. A specified culture temperature can be kept throughout the fermentation by manipulating the bioreactor jacket's hot and cold water flow rates.

2.2. P&ID

The P&ID of the controlled system is shown in Fig. 1. The model considers that cultivations are carried out in a 5 L jacketed bioreactor (R-001). Crude glycerol (stream 1) is fed into the system through a peristaltic pump P-001. A water stream coming from two sources circulates through the jacket, one at 20 °C (stream 2) and another at 80 °C (stream 3); these streams were driven by pumps P-002 and P-003, respectively. Finally, the model considers a level alarm where an on/off switch manipulates the bioreactor feed. When a defined maximum level is reached, the alarm turns off the feed pump ending the process.

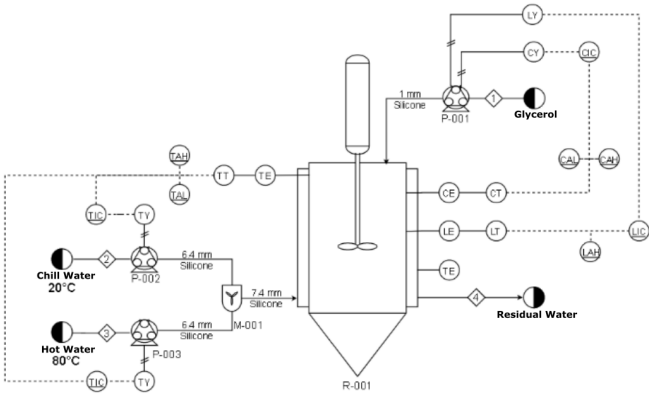

Figure 1. P&ID diagram of the bioreactor.

2.3. Mathematical model

The model was derived from mass and energy balances assuming the following:

- Perfectly agitated bioreactor and jacket.
- No heat losses to the environment.
- Constant physical properties.

2.3.1. *Variables*

The input variables to the bioreactor are the glycerol inlet concentration, jacket inlet temperature, glycerol flow rate and jacket inlet flow, the last three are the manipulated variables. The model consider two measured external disturbances, the first one over the make-up waters temperature due to the difference between those and the ambient temperature, and the second one over the concentration of glycerol. There are 7 state variables, volume of bioreactor, concentration of biomass, concentration of 1,3-Propanodiol, concentration of acetic acid, temperature of the jacket, concentration of glycerol and temperature of the reactor, the last two are also the measured outputs used for the control loops.

2.3.2. *Mass balances*

The equations modeling the fedbatch bioreactor were derived based on different existing models in the literature (Kaur et al. (2012b) and Silva et al. (2015)) and as an innovative factor, a temperature dependence was added to maximize 1,3-PD production. The biomass evolution is determined by its specific growth rate μ

and the bioreactor's dilution rate D:

$$\frac{dX}{dt} = \mu X - DX \tag{1}$$

Where X corresponds to the biomass concentration in the reactor in g/L. For glycerol (S), the mass balance involves its concentration in the inlet stream and its specific consumption rate q_S multiplied by the biomass concentration:

$$\frac{dS}{dt} = -q_S X + D(S_{in} - S) \tag{2}$$

Both product (P) and acid (A) concentrations are determined by their specific production rate (q_P and q_A) multiplied by X plus the dilution term:

$$\frac{dP}{dt} = q_P X - DP \tag{3}$$

$$\frac{dA}{dt} = q_A X - DA \tag{4}$$

Finally, the reactor volume (V) is determined by the glycerol inlet feed rate (F):

$$\frac{dV}{dt} = F \tag{5}$$

2.3.3. Energy balances

For the energy balance of the cultivation media within the bioreactor, we consider: i) the temperature of the inlet streams (glycerol and cooling water), ii) the heat reaction term associated to 1,3-PD production, and iii) the heat exchange with the cooling jacket:

$$\frac{dT_r}{dt} = D(T_{in} - T_r) + \frac{q_P X \Delta H_r}{32 \rho_r C_{heat,r}} - \frac{K_T A_T (T_r - T_j)}{V \rho_r C_{heat,r}} \tag{6}$$

T_{in} and T_j correspond to the inlet temperatures of the feed stream and cooling water, respectively, and T_r to the cultivation media temperature. H_r is the product enthalpy of formation, and ρ_r and $C_{heat,r}$ are the density and the specific heat of the the cultivation media, respectively. K_T and A_T are the overall heat transfer coefficient and the heat transfer area between the cultivation media and the cooling water, respectively. The jacket energy balance considers the temperature difference between the inlet and outlet temperature of the cooling water, and the heat exchanged with the cultivation media,

$$\frac{dT_j}{dt} = \left(\frac{F_j}{V_j}\right)(T_{j,in} - T_j) + \frac{K_T A_T (T_r - T_j)}{V_j \rho_j C_{heat,j}} \tag{7}$$

F_j and $T_{j,in}$ correspond to the flow rate and inlet temperature of the cooling water, respectively, V_j is the jacket volume.

2.3.4. Constitutive equations

The specific growth rate (μ) is described by an Arrhenius temperature dependant model:

$$\mu = \mu^* \frac{\left(k_g exp\left(-\frac{E_g}{R((T_r+\theta)+273.15)}\right) - k_d exp\left(-\frac{E_d}{R((T_r+\theta)+273.15)}\right)\right)}{\omega} \tag{8}$$

θ and ω are constants that shift the Arrhenius function so that its maximum growth is at 37 °C as reported in Junghare et al. (2012). R is the gas constant, and E_g and E_d are the activation energies for growth and death, respectively. Besides, μ is determined by a growth inhibitory term (μ^*), which indicates that after a certain threshold, defined for S, P, and A, biomass generation is inhibited.

$$\mu^* = \frac{S \cdot \mu_{max}}{S + K_S}\left(1 - \left(\frac{S}{S_m}\right)^a\right)\left(1 - \left(\frac{P}{P_m}\right)^b\right)\left(1 - \frac{A}{A_m}\right) \tag{9}$$

The parameter K_S corresponds to the saturation constant for the substrate, a and b are dimensionless parameters of the model, and Z_m with $Z = S, P, A$ corresponds to the maximum inhibitory growth threshold for each compound. The consumption and generation rates have both growth and non-growth (maintenance) associated terms,

$$q_S = \left(\frac{1}{Y_{mS}}\right)\mu + m_S \tag{10}$$

$$q_P = K_1 \mu + K_2 \tag{11}$$

$$q_A = \left(\frac{1}{Y_{mA}}\right)\mu + m_A \tag{12}$$

K_1 and K_2 are growth constants, Y_{mS} and Y_{mA} correspond to maximum yield rates with respect to the substrate, and finally m_S and m_A are maintenance energy constants. The dilution factor D is defined as:

$$D = \frac{F}{V} \tag{13}$$

For the cooling water stream, which comes from a hot and a cold source, a simple energy balance yields:

$$F_j = F_{cold} + F_{hot} \tag{14}$$

$$T_{j,in} = \frac{F_{cold} T_{cold} + F_{hot} T_{hot}}{F_j} \tag{15}$$

F_{cold} and T_{cold} correspond to the flow rate and temperature of the cold source, and F_{hot} and T_{hot} correspond to the flow rate and temperature of the hot source.

2.3.5. *Initial Conditions*

The initial conditions of the simulation are: X_0 = 2 g/L, S_0 = 1 g/L, P_0 = 0 g/L, A_0 = 0 g/L, V_0 = 1.5 L, T_{r_0} = 25°C and T_j = 25°C.

2.4. Optimum path for the process

In order to maximize the concentration of 1,3-PD in the reactor, the Orthogonal Collocations on Finite Elements (OCFE) procedure by Carey and Finlayson (1975) was implemented, which allows us to calculate optimal trajectories for the manipulated variables.

This method allows the differential algebraic equation (DAE) system to be completely discretized, approximating the control variables and the states (x) by polynomial functions (Ω) at each time interval (finite element).

The states are defined as:

$$\left.\begin{aligned} x(t) &= x_{i-1} + h_i \sum_{j=1}^{cp} \Omega_j\left(\frac{t - t_{i-1}}{h_i}\right)\frac{dx}{dt}_{i,j} \\ x_i &= x_{i-1} + h_i \sum_{j=1}^{cp} \Omega_j(1)\frac{dx}{dt}_{i,j} \end{aligned}\right\} \quad t \in [t_{i-1}, t_i] \tag{16}$$

t_{i-1} and t_i represent the begining and end of the time interval (h_i) of finite element i. Three Radau collocation points (τ_j) where used, 0.155051, 0.644949 and 1 respectively. With this process it was able the discretization of the states and the control variables.

The formulation of the dynamic optimization problem is the following:

$$\begin{aligned} \max_{\mathbf{u}} \quad & P(t_f) \\ & \frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x},\mathbf{y},\mathbf{u},t) \\ & \mathbf{x}(t_0) = \mathbf{x}_0 \\ & \mathbf{y} = \mathbf{g}(\mathbf{x},\mathbf{u},t) \end{aligned} \tag{17}$$

Where $P(t_f)$ is the concentration of 1,3-PD in the bioreactor at the final time, $\mathbf{x}$ is the differential state variables vector, $\mathbf{y}$ the algebraic state variables vector and $\mathbf{u}$ the input (control) variables vector which are the substrate flow-rate and hot and cold water flow-rate. To solve this dynamic optimization problem, the software AMPL was used where an optimum substrate trajectory is obtained in the reactor, which will be used as a set-point to control the process.

2.5. Multi-Variable Model Predictive Control

The Model Predictive Control Toolbox from MATLAB R2021a was used to design the MPC controller, where the transfer functions used on the design were the ones determined previously. The considered cost function was,

$$\begin{aligned} J = &\sum_{j=1+d}^{N_p+d} \delta(j)\,[\hat{y}(t+j \mid t) - w(t+j \mid t)]^2 \\ &+ \sum_{j=1}^{N_c} \lambda(j)\,[\Delta u(t+j-1)]^2 \end{aligned} \tag{18}$$

N_p and N_c represent the prediction and control horizon, which were 20 and 10 respectively. The sample time was set to 0.025 h. δ and λ represent the weights for the reference and control action, which were set to 6.82 and 0.48, respectively. The weight of the control action rate was set to 0.048. The control action indicates how many revolutions per minute at which each pump operates, and it has a range of 0-600 RPM. The maximum rate for the hot water pump was restricted to 20 RPM per hour in order to avoid a large overshoot in the temperature set point. These values were obtained by trial and error to achieve a good controller performance. The minimization problem is represented by,

$$\begin{aligned} \min_{\mathbf{u}} \quad & J \\ & \mathbf{u} \geq 0 \\ & \mathbf{S},\mathbf{P},\mathbf{A},\mathbf{X} \geq 0 \end{aligned} \tag{19}$$

Where $\mathbf{u}$ is the control action that corresponds to the revolutions per minute at which each pump operates (F, F_{cold}, F_{hot}). On the other hand, $\mathbf{S}$, $\mathbf{P}$, $\mathbf{A}$ and $\mathbf{X}$ are the concentration of substrate, product, acid, and biomass, respectively.

2.6. Results

A simulation was performed using *Simulink*, using the MPC to control both the substrate and the reactor temperature. The following figures show the obtained results for the controlled and manipulated variables of the system.

2.7. Discussion

The optimal trajectory obtained by AMPL (Fig. 2) is consistent with the behavior of the microorganism. In the initial phase of the culture, a high glycerol value is obtained due to the necessity of increasing the biomass concentration as fast as possible, followed by a progressive decrease in the substrate concentration since is also inhibitory for the growth of the organism. After 25 hours the substrate is kept close to zero to maximize

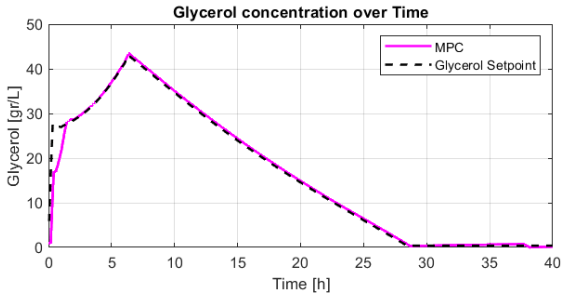

Figure 2. Controller behavior with variable substrate setpoint

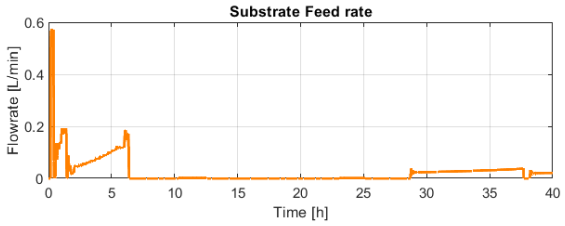

Figure 3. Substrate pump behavior

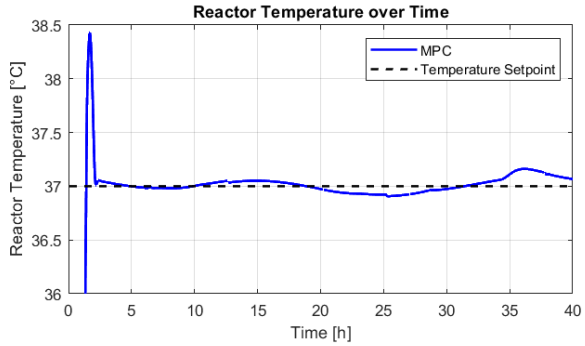

Figure 4. Controller behavior with given temperature setpoint

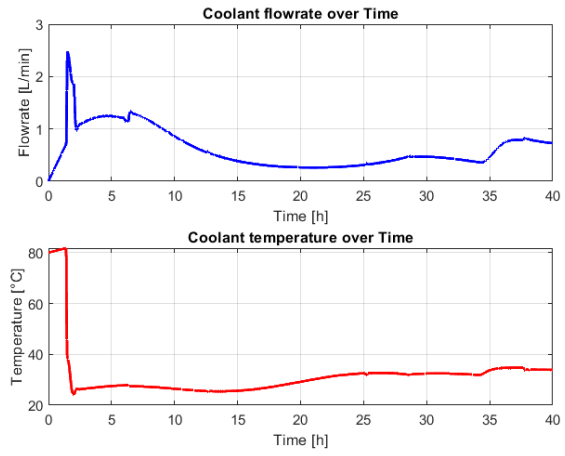

Figure 5. Inlet Flowrate and temperature of Coolant

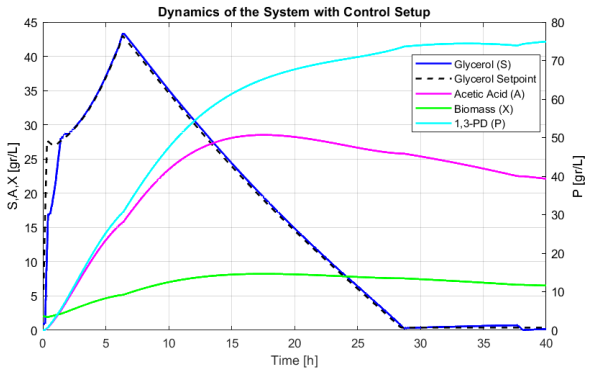

Figure 6. Complete dynamics of relevant system state variables

the production of 1,3-PD.

The predictive model controller was able to accurately follow the optimal trajectory obtained with the optimization using AMPL. Despite the disturbances, the MPC manages to control successfully. Fig. 3 shows the behavior of the substrate pump, where the pump reaches its saturation value in the first hour due to the need to increase the substrate concentration quickly. After 6 hours the pump activity is kept to a minimum to allow the microorganism to consume the substrate. Finally, after 25 hours the pump operates feeding a minimum amount of substrate so that the microorganism continues to produce 1,3-PD without being inhibited by its concentration.

Figure 4 shows the reactor temperature over time, where the rapid increase in temperature from the initial temperature to the setpoint can be seen. An overshoot is obtained when the temperature reaches the desired value, which was minimized using the Model Predictive Control Toolbox of MATLAB. It can be seen that the controller is able to maintain the reactor temperature close to 37 °C despite disturbances in the coolant sinks.

The coolant flow and its temperature can be seen in Figure 5, where a peak in the flow can be seen due to the need to decrease the reactor temperature to avoid an overshoot. Due to this, there is a sharp drop in the inlet temperature. After this, the controller keeps varying the flow and temperature according to the perturbations and the heat released by the reactor.

The complete dynamics of the reactor state variables is shown in Fig. 6. Where a value of approximately 75 g/L of the desired product is reached (this being the major compound produced), while the concentration of acetic acid, substrate and biomass remain relatively low.

3. Conclusions

In this work the implementation of a predictive control model for the production of 1,3-Propanediol from glycerol by Clostridium butyricum was simulated using *Simulink*. Since the fermentation substrate is also

inhibitory for the microorganism, an optimal feeding strategy was proposed, which served as a variable setpoint for the MPC. The implemented controller was able to successfully maintain both the substrate and the reactor temperature at the desired values, resulting in a final concentration of 74.94 g/L of 1,3-PD. It should be noted that although the control strategy with optimized variable setpoint obtained higher product values compared to the use of a fixed optimized setpoint used in a previous work, this gain does not constitute a significant increase. This is mainly due to the yield parameters of the mathematical model that prevent obtaining a higher concentration. However, the technique developed in this work could be extremely valuable for increasing the productivity of other inhibitory growth fermentation processes.

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