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ENHANCING pH CONTROL IN A BIOREACTOR THROUGH EXPERIMENTAL SYSTEM IDENTIFICATION AND DYNAMIC ANALYSIS

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Highlights

- Models pH behavior in fermentation using dynamic analysis and system identification
- Compares FOPDT and second-order ARMAX models for pH control accuracy
- The ARMAX model gives a more precise system behavior prediction than the FOPDT
- Methods support improved control and optimization in bioprocess applications

Abstract

The acidic by-products produced during fermentation can cause a drop in pH, which in turn affects the microorganisms' growth and the product's formation. In order to keep pH at the desired level, process control becomes necessary. The aim of this study is to develop a predictive model for pH behavior during the fermentation of Clostridium acetobutylicum through dynamic analysis and system identification. The First Order Plus Dead Time (FOPDT) model and the second-order Autoregressive Moving Average with Exogenous (ARMAX) model were the two approaches that were compared. While the FOPDT model was used to derive the PID controller parameters through transient analysis, the Smith and linear regression methods, the ARMAX model–identified with the Recursive Least Squares (RLS) method–was chosen for its better accuracy in capturing input-output dynamics. PID tuning was done with the Cohen-Coon method. The simulation results showed that setpoint tracking was successfully done, and the ARMAX model provided a more accurate representation of the system. The optimized PID controller recorded the minimum Integral of Squared Error (ISE) value of 50.82. This study points out effective modeling and control strategies for the production of stable pH during fermentation, thus providing very useful knowledge for other bioprocesses that require precise control.

Keywords: dynamic analysis, system identification, FOPDT model, ARMAX model, theoretical PID control.

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INTRODUCTION

The operational dynamics of bacterial fermentation processes are of great importance for allowing microorganisms to grow and for the formation of new products. The fermenting conditions can be varied considerably, and this will definitely influence the efficiency of the fermentation process. One of the main reasons for that is the product or by-product build-up in the growth medium during the fermentation process, which usually results in lower productivity and reduced yields. The pH factor is the most important one in this case because it controls the fermentative metabolism through the regulation of the activity of the extracellular enzymes on the substrates. Moreover, the surrounding conditions and the kinetics and thermodynamics of accompanying redox reactions are involved in the fermentation process control [1].

Anaerobic fermentation, which refers to the process of fermentation performed in the total absence of any external electron acceptor, requires the organic substrate to be partially oxidized to maintain the balance of electrons that are being reduced to hydrogen and other products like ethanol and short-chain fatty acids. Thus, the bacterium's acid-producing activity results in a drop of pH and the subsequent decline of microbial growth and product output. In addition, the pH conditions affect the ratio of the fermentation product with regard to the different kinds of compounds (e.g., hydrogen, volatile acids, solvents) that

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have their highest production at certain pH levels.

For instance, in certain *Clostridial* species performing acetone-butanol-ethanol (ABE) fermentation, a pH shift from 6.0 to 4.5 triggers a transition from acidogenesis to solventogenesis [2,3]. This suggests that pH control is a key strategy for optimizing solvent production [2,3], though some studies report successful solvent generation even without active pH regulation [4]. Similarly, in syngas fermentation by *Clostridium carboxidivorans*, maintaining pH at 5.75 or higher promotes acid accumulation, which in turn enhances alcohol synthesis in hexanol-butanol-ethanol (HBE) fermentation [5].

Likewise, in biohydrogen production, the accumulation of volatile fatty acids during fermentation leads to pH reduction, significantly inhibiting hydrogen yield [6]. Various experimental approaches have been explored to mitigate the adverse effects of pH and optimize product yields. These methods have been applied to both simple fermentation systems, such as dark and photofermentation, as well as more complex setups, including two-stage systems where dark fermentation is followed by photofermentation [7,8] or systems involving different bacterial strains in combination [9,10]. Other advanced strategies include microaerobic dark fermentation [11] and microbial electrolysis following dark fermentation [12,13]. All of these approaches can benefit from improved process control methodologies.

Process control strategies rely on understanding system behavior in response to input changes or disturbances to develop controllers that regulate key process variables effectively. By employing dynamic analysis and system identification, an accurate model that describes system behavior can be determined, providing valuable insights into process dynamics [14,15]. In other words, effective control enhances productivity, quality, and yield, largely depending on the accuracy of the system model [14,16,17].

A commonly used empirical model for capturing input-output dynamics in many processes is the First Order Plus Dead Time (FOPDT) model, which represents a first-order system with time delay. The parameters derived from this model serve as a foundation for tuning proportional-integral-derivative (PID) controllers [17]. The FOPDT model provides a reliable approximation of bioprocess behavior, aiding in controller parameter tuning during process optimization and control simulations. Its parameters can be determined through methods such as graphical transient analysis [19], Smith approximation [20], and linear regression [21]. Moreover, since bioprocess control relies on the selection of process models, control parameters, and control algorithms, advanced system identification techniques utilizing both linear (ARX, ARMA, ARMAX, ARARX, ARARMAX) and nonlinear (NARX, NARMX) models can be beneficial [22].

System identification refers to the development of mathematical models from input-output data obtained using various signal types, such as step, square wave, ternary, or random inputs. Several identification methods, including genetic algorithms, Levenberg-Marquardt optimization, least squares (LS), Biermann's method, and recursive least squares (RLS), have been employed for model parameter estimation [23]. Due to its effectiveness in capturing system dynamics, the Autoregressive Moving Average with Exogenous input (ARMAX) model has been widely applied in system identification studies [14,23-27].

Some of the newer technologies in bioprocess control have centered around employing experimentally-derived system identification data mixed with novel controls to offer improved process stability, efficiency, and scalability. Techniques such as model predictive control (MPC), adaptive PID control, and hybrid data-driven models that merge mechanistic (first-principles) models with machine-learning features have shown tremendous potential for optimizing fermentation processes [28-30]. In parallel, recent surveys in parametric identification demonstrate robust ARMAX estimation techniques as well as demonstrate the importance of accounting for non-Gaussian and coloured noise in identifying dynamic models from experimental data [31]. Reviews of PID, fuzzy, and hybrid PID schemes further emphasize pragmatic strategies for improving closed-loop performance in nonlinear chemical and bioprocess systems [32]. As these works underpinning experimentally validated dynamic modelling ensure the specificity of intelligent process control, they entail both software development and integration with experimental rig development. Despite these advances, few experiments have produced control-oriented, experimentally determined pH models of bio-reactors useful directly for controller design and theoretical controller performance assessment. This work addresses this by experimentally measuring pH dynamics in a *Clostridium acetobutylicum* fermentation and comparing FOPDT- and ARMAX-based modeling approaches to assess and optimize theoretical controller performance.

In this study, a rigorous approach is presented to enhance pH regulation in *C. acetobutylicum* fermentation through the combination of experimental dynamic analysis with system identification. Motivation for this study comes from the critical need for pH regulation in maintaining microbial activity, product yield, and process stability in anaerobic fermentations. Despite extensive work devoted to the optimization of fermentation, few studies have integrated experimental evidence with a model-based optimal control strategy optimized for bioprocesses characterized by the presence of intrinsic nonlinearity and time delay. This paper provides an attempt at filling the gap through the development and comparison of two modeling paradigms: the FOPDT model and the ARMAX model, both derived from experimental input-output data. The FOPDT model can facilitate PID controller tuning using classical methods, while the ARMAX model allows for a higher-order model that can be used to model complex pH dynamics. Through the integration of these two methods, the current study creates a sound theoretical framework for model-based pH control that can be developed for various bacterial fermentation processes. The novelty of this work is the experimental determination of process dynamics from open-loop bioreactor data and their integration into theoretical control assessment. This study differs from previous research, which relied on simulated data or linearized models alone, by establishing a data-driven approach linking empirical bioprocess behavior with control system design. The work thus provides an experimentally tested, theory-based contribution toward future model-based and adaptive control technology in fermentation engineering.

MATERIALS AND METHODS

Growth Medium

The solvent-producing anaerobic bacterium *C. acetobutylicum* NRRL B-527 was grown on potato extract as the medium for fermentation. The procedure of preparation included the peeling and cutting of the potatoes, which were then extracted through a juicer. The extract was diluted with bi-distilled water and subjected to boiling for half an hour, thus helping the conversion of starch into glucose. The boiled suspension was then transferred into a glass bottle with a working volume of 500 mL, and its pH was adjusted to 6 using 0.1 M H_2SO_4 and/or 0.1 M NaOH solutions [33]. To establish anaerobic conditions, the sealed bottle was autoclaved at 121 °C and 1.2 atm for 20 minutes (ALP, CL40M) and subsequently purged with nitrogen.

Inoculation

A tube containing 5 mL of growth medium would be incubated at 28 °C for 24 hours after 1 mL of the culture medium was inoculated. Then, 5 mL of the incubation culture was transferred into an anaerobic bottle, which contained 500 mL of the medium, and again incubated at 28 °C for 24 hours. At last, 500 mL of this inoculated culture was added to the bioreactor containing 5 L of growth medium.

Bioreactor

The bioreactor (Techfors S, Infors HT) consisted of a thermocouple, sparger, impeller, and microbiological filter. The concentration of dissolved oxygen in the growth medium was continuously monitored by a probe (WTW, pH340i), and a pH probe (WTW, pH340i) was provided for pH measurement. Acid and base solutions were supplied by peristaltic pumps (Peristar, USA), and a steam generator was available for sterilization. Data acquisition modules (Commat Instrument Company, PCM 9901) gathered process data that was logged online via a computer. The readings of pH, dissolved oxygen, and thermocouple were transmitted to the computer with the help of IRIS V 5.2 software. Nitrogen gas was sparged into the growth medium to create anaerobic conditions.

Dynamic Analysis and PID Control

In order to build a system model and control the process, the relationship between input and output variables must first be established. The manipulated variable is the input variable that directly affects the output and is, hence, adjusted; the controlled variable is the one that is chosen because of its major impact on the product yield. The process can be mapped by monitoring the fluctuations of the controlled variable over time and altering the input to find the correlation between them. System identification methods can be employed to replicate the system with experimental input-output data as a foundation [14].

Given its critical role in fermentation, pH was chosen as the controlled variable in this study [33]. The basic flow rate of the liquid and the peristaltic pump speed were used as the input and manipulated variables, respectively. The dynamic analyses were conducted through transient, Smith, and linear regression techniques to identify the FOPDT model parameters by applying various step inputs. The obtained model was then used to compute the control parameters for the PID algorithm employing the Cohen-Coon method. To further understand the pH behavior more thoroughly, the parameters of a second-order ARMAX model were determined through the RLS method, utilizing more sophisticated inputs like square waves, random signals, and ternary signals. At last, the theoretical PID control simulations relying on the ARMAX model were conducted to measure the theoretical pH control efficiency. Data utilized in this study were gathered from an experimental study conducted under open-loop control of the bioreactor to observe the natural variation of pH during the fermentation. During this phase, the reactor was operated without feedback control to record the interaction between the controlled variable (pH) and the manipulated variable (flow rate of the base). The input-output data so generated were subsequently utilized to identify the process dynamics through FOPDT and ARMAX modeling. These system identification experiments were complemented by theoretical pH control experiments utilizing the models developed for evaluating the performance of the proposed control schemes. This theoretical control step was executed in a simulation environment to validate the controller design before implementing it in real time, in accordance with standard model-based control development practices. This approach maintained all of the modeling and simulation tasks grounded in experimentally observed process behavior with a minimum of experimental complexity and bioreactor safety in the early stages of evaluation.

A diagram of the MATLAB™/Simulink® interface developed for real-time monitoring and control simulations is presented in Figure S1 (Supplementary Material). The illustration depicts data communication between the bioreactor instrumentation and the control module, and how input-output signals were recorded and processed during the dynamic analysis. This model captures pH readings and generates the necessary flow rate signals, as illustrated in Figure S1. The process reaction curve was obtained by applying various positive and negative step responses, and the FOPDT model, represented by Eq. 1, was used to determine the model parameters. In this equation, Kp represents the process gain, θ denotes the dead time, and τ is the process time constant. The model parameters for the FOPDT system were determined using transient analysis, Smith, and linear regression methods.

$$Gp(s)=\frac{K_p}{\tau s+1}e^{-\theta s} \tag{1}$$

Transient Analysis Method: After obtaining the process response curve of the system, the FOPDT model parameters can be determined by identifying the optimal tangent at the curve's turning point, as illustrated in Figure S2a [19].

Smith Method: The model parameters are calculated using Figure S2b and Eq. 2 [20]. In these calculations, the applied step change to the input (δ), the difference between the first and second steady-state values of the output variable (Δ), and the time taken to reach 28% ($t_{28\%}$) and 63% ($t_{63\%}$) of the second steady-state value of the output variable are considered.

$$K_p = \frac{\Delta}{\delta}$$
$$t_{28\%} = \theta + \frac{\tau}{3}$$
$$t_{63\%} = \theta + \tau$$
$$\tau = 1.5\left(t_{63\%} - t_{28\%}\right)$$
$$\theta = t_{63\%} - \tau \tag{2}$$

Linear Regression: Eq. (3) shows the FOPDT system's reaction to a step input of amplitude (A) supplied to the system using the acquired experimental input-output data.

$$y(t) = AK_p\left(1 - e^{\frac{\theta - t}{\tau}}\right) \tag{3}$$

where $y(t)$ is the process output at time t; A is the applied step-input amplitude; K_p is the process gain; θ is the process dead time; τ is the time constant; and e is the base of the natural logarithm.

If Eq. (3) is linearized, it yields Eq. (4) based on [34]. The calculations are shown in Figure S2c.

$$\ln\left(1 - \frac{A}{K_p}\right) = -\frac{t}{\tau} + \frac{\theta}{\tau} \tag{4}$$

where A is the step-input amplitude, K_p is the process gain, t is time, τ is the time constant, and θ is the process dead time.

The FOPDT model parameters obtained were used with the Cohen-Coon Method to calculate the PID control parameters, where K_c is the proportional gain, π is the integral, and τ_D is the derivative given in Eqs. (5)-(7) [34].

$$K_c = \frac{1}{K}\frac{\tau}{\theta}\left[\frac{4}{3} + \frac{\theta}{4\tau}\right] \tag{5}$$

$$\tau_I = t_d\left[\frac{32 + 6\theta/\tau}{13 + 8\theta/\tau}\right] \tag{6}$$

$$\tau_D = t_d\left[\frac{4}{11 + 2\theta/\tau}\right] \tag{7}$$

Further studies were carried out by giving advanced inputs to the manipulated variable and choosing a more complex model, such as the ARMAX model, Eq. (8), and applying the RLS algorithm. The detailed system identification procedure is given in previous reports [37-39].

$$\begin{aligned} &y(t) + a_1 y(t-1) + \ldots + a_{n_a} y(t-n_a) = \\ &= b_0 u(t-1) + \ldots + b_{n_b} u(t-n_b) + e(t) \end{aligned} \tag{8}$$

where $y(t)$ is the output variable, $u(t)$ is the input variable, and $e(t)$ is white noise.

A second-order ARMAX model was selected, and the model parameters (a_1, a_2, b_0, and b_1) were calculated using the RLS method with a 0.96 forgetting factor and a 1000 initial value of the covariance matrix to obtain a suitable model.

RESULTS AND DISCUSSION

Open Loop Operation of the Bioreactor

The IRIS V.2 software helped to keep track of pH changes in the course of nine hours of fermentation of *C. acetobutylicum*. The pH was taken as the output variable, and its variations over time (open-loop behavior) were noted under the stable operating conditions of 37 °C and 600 rpm stirring speed. The fermentative process resulted in the production of volatile fatty acids, which caused the pH to drop within the range of 5.7 to 4.7 (Figure 1). Figure 1 illustrates the pH profile observed during the course of batch fermentation. The drop in pH initially is a result of the metabolic action of *C. acetobutylicum* during the acidogenic phase, where the microorganism catabolizes carbohydrates into organic acids such as acetic and butyric acids. This build-up of acid lowers the pH progressively and, if left uncorrected, can hamper enzymatic activity, slow down cellular growth, and ultimately reduce solvent productivity efficiency. Therefore, pH drop is a critical indicator of metabolic shift in the culture. pH must be maintained in a narrow optimum range to ensure the equilibrium between acid formation and solvent formation and prevent irreversible shifts to inhibitory values. Such behavior is interpreted to be the background for the motivation of the present research, emphasizing the necessity of an effective control strategy capable of stabilizing pH and delivering reliable fermentation performance.

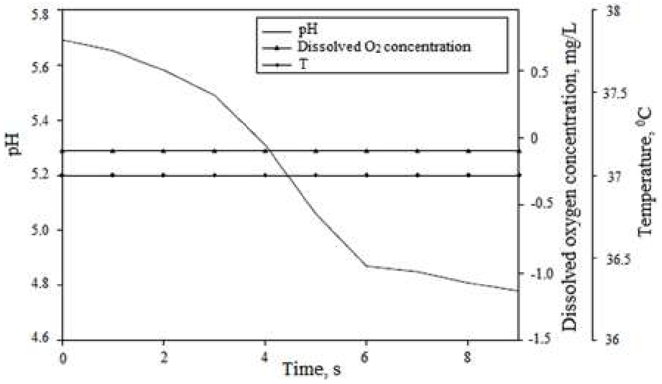

Figure 1. The pH drop that occurs during dark fermentation without pH control (open loop operation), with the growth medium's temperature and dissolved oxygen concentration remaining constant.

Selection of the Manipulated Variable and Determination of Acid-Base Pair Concentration

The H_2SO_4-NaOH acid-base pair was chosen for pH regulation during fermentation. The concentration of the acid and base was determined based on the guideline that the total volume of added acid or base should not exceed 10% of the overall reaction volume [35].

Preliminary studies were conducted to identify acid and base concentrations that could replicate the pH decline observed in open-loop operation. These studies helped establish the appropriate concentrations and volumes of acid and base to be used in dynamic and control experiments. Small incremental volumes of 0.5 M, 0.1 M, 0.05 M, and 0.01 M sulfuric acid solutions were added to 500 mL of medium, and the resulting pH drop was recorded (Figure S3).

Figure S3 displays the results of the first experiments conducted to test the acid concentration neutralization capability prior to system identification. These experiments were conducted to study the dynamic behavior of the fermentation medium in response to stepwise addition of acid and select a proper acid concentration that is capable of bringing about observable but stable pH changes. The motivation behind this analysis was to ensure that the induced pH changes were within a reproducible and safe range of operation, avoiding abrupt spikes that would disturb microbial metabolism or impair instrumentation. The selected concentration provided an optimal compromise between responsiveness and system stability and thus constituted the experimental foundation for finding the range of manipulated variables included in the follow-up modeling and control examinations. This early study was crucial in developing meaningful dynamic tests and confirming the validity of the discovered pH models. The acid concentration that produced a pH decline similar to that observed in open-loop bioreactor experiments was identified. Based on Figure S3, 0.1 M H_2SO_4 was determined to be the most suitable concentration.

To obtain a reliable process reaction curve—an essential step in dynamic model identification—the system needed to operate at a steady state [20]. The objective of this study was to determine the steady-state acid and base flow rates necessary to maintain optimal pH levels throughout fermentation.

Experiments with varying base flow rates were conducted using 0.1 M H_2SO_4 at a fixed flow rate of 0.081 mL/min, simulating the pH drop in the growth medium. The optimal NaOH concentration was identified as the one capable of neutralizing H^+ ions and compensating for the pH decrease observed in the open-loop process.

Determination of Optimal NaOH Concentration

In order to determine the ideal concentration of the base that may be employed to regulate pH, a series of open-loop experiments was conducted using NaOH solutions of varying molarities. Each of the different concentrations was subjected to identical operating conditions to observe the pH response to successive additions of base. The objective was to determine a concentration from which pH could be adjusted efficiently, with little overshooting and system stability. Comparative analysis of the pH response curves showed that intermediate concentrations of NaOH produced the most stable and reproducible range of control, except for the rapid pH oscillations observed at high concentrations. Based on these results, the selected concentration was employed in subsequent dynamic testing and control design.

Determination of Steady State

To identify the steady-state operating point, the base flow rates were experimentally tested in open-loop conditions. Of the flow rates tested, 0.085 mL/min was identified as the optimum for stable pH with minimal fluctuation. This was selected as it had the least pH variation with time and had constant and reproducible system performance. Increase in base flow rates induced immediate pH overshoots and nonlinear response patterns, while flow rate reductions produced sluggish correction dynamics and extended stabilization times. The chosen rate of 0.085 mL/min was therefore an optimal balance between steady-state stability and responsiveness, ensuring the quality of dynamic modeling and the reliability of adherence to system identification procedures.

Figure 2 illustrates the experimentally measured pH response of the *C. acetobutylicum* fermentation system during open-loop operation for a step NaOH input signal. The pH is initially decreased due to acid production during the initial acidogenic stage and then increases slowly following base addition. The resulting response curve is typical of first-order dynamic behavior with an apparent time delay between the input disturbance and observable pH change. Even with the lowest base flow rate, a steady state could not be achieved with 0.5 M NaOH and flow rates of 0.1875 and 0.085 mL/min, as shown by the pH fluctuations (Figure 2(a)). This was due to the base concentration being too high. However, pH stability was maintained at a flow rate of 0.085 mL/min using 0.3 M NaOH (Figure 2(b)). To verify these results, a second experiment was conducted where pH variations were monitored under different base flow rates, with the bioreactor conditions simulated using a constant acid flow rate of 0.081 mL/min. It was confirmed that maintaining a stable pH during fermentation was achievable when using 0.3 M NaOH at various base flow rates (Figure 2(c)).

The determination of a steady base flow at 0.085 mL min^{-1} ensured stable operation during identification experiments. At this flow rate, minimal oscillation and a standard deviation below 0.02 pH units were observed, confirming an adequately steady baseline. The derived process parameters indicate that the dynamic pH behavior can be well approximated by an FOPDT structure for initial controller design.

Input - Output Relations for Obtaining the Process Reaction Curve and Determination of FOPDT Model Parameters

To determine reliable controller settings, step inputs were applied to the process under various operating conditions. The model parameters for each resulting reaction curve were then estimated using transient analysis, Smith, and linear regression techniques. Here, the word "reaction" has reference only to "reaction curve," meaning the open-loop dynamic response of the system to a specified step change in the input variable, i.e., addition rate. The curve tells us about the change in output with time and is the experimental basis on which process parameters - gain (*Kp*), time constant (τ), delay time (θ) - are established. Reaction curve characterization provides the required quantitative input-output signal relationship, which serves as the basis for the FOPDT and ARMAX model identification and subsequent control analysis.

Different base flow rates were employed for NaOH solutions with varying concentrations to supply the same hydroxide ion addition and create the same titration effects in the experiments. Since the neutralization capability of NaOH is concentration and volumetric flow rate-dependent, slower flow rates were employed for more concentrated solutions (0.5 M) to prevent pH overshoot. Less concentrated solutions (0.3 M) required somewhat higher flow rates to achieve the same extent of pH change. This design strategy supplied the system with the same total molar quantities of base per unit time in every test so that the dynamic pH responses could be meaningfully compared and the process model parameters reliably established under controlled and reproducible conditions.

Figure 3 shows the corresponding process reaction curves obtained under different base addition rates. These data were used to estimate the process parameters - process gain (*Kp*), time constant (τ), and time delay (θ) - for both with microorganism and without microorganism conditions. As summarized in Table 1, the system containing microorganisms exhibits a smaller effective gain and a longer time delay, reflecting the buffering effects of microbial metabolism and the slower response of biological activity to pH perturbations.

When the system operated at a steady state with the target pH of 5.2, the first reaction was observed following a positive step input, which increased the flow rate of 0.3 M NaOH from 0.087 mL/min to 0.4581 mL/min (Figure 3(a)).

Additional experiments were carried out with a different setup. A 2 L bioreactor was used under anaerobic conditions with an agitation speed of 600 rpm, a pH of 6.1, and a temperature of 37 °C. The base concentration was set at 0.3 M, and a step input was applied, increasing the flow rate from 0.285 mL/min to 0.5737 mL/min (Figure 3(b)).

Two separate reaction curves from Figure 3, along with transient analysis, Smith, and linear regression methods, were used to determine the FOPDT model parameters after obtaining the reaction curves without and with microorganism from the input-output relationships (Table S1).

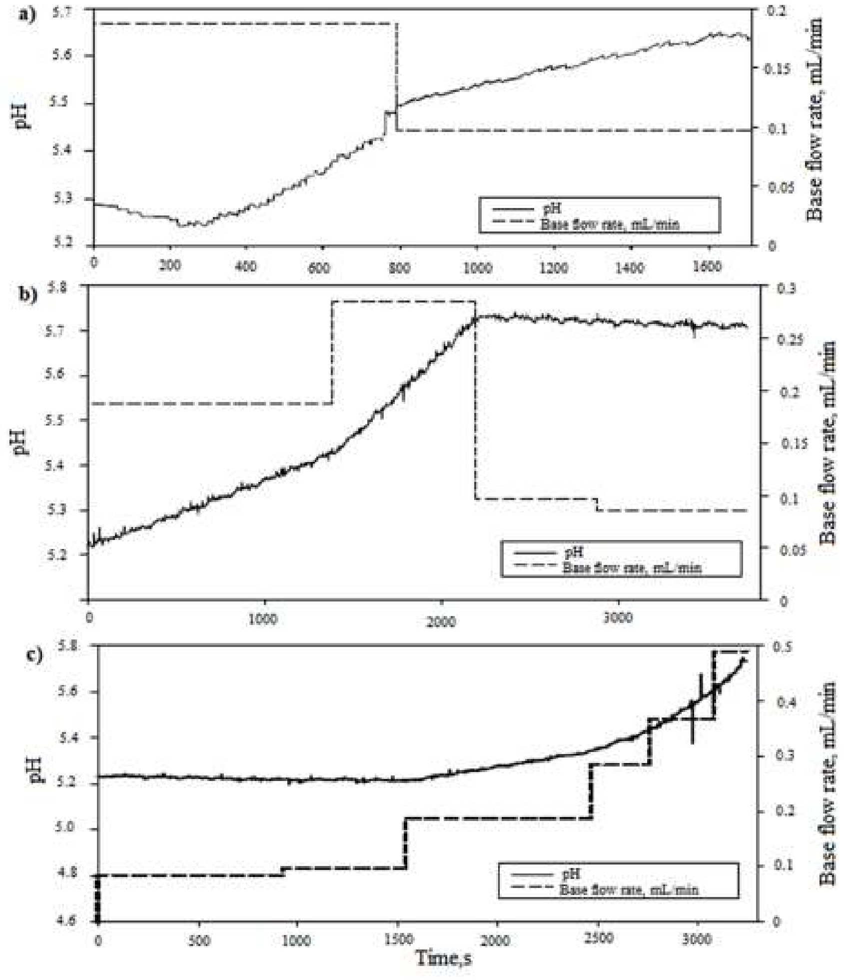

Figure 2. pH changes according to different base flow rates with constant acid flow rate at 0.081 mL/min a) 0.5M NaOH with 0.185 and 0.085 mL/min base flow rates, b) 0.3 M NaOH with 0.185, 0.285, 0.1, and 0.085 mL/min, and c) simulation of bioreactor conditions with desired value of pH at 5.2 by 0.081 mL/min constant acid flow rate and various base flow rates.

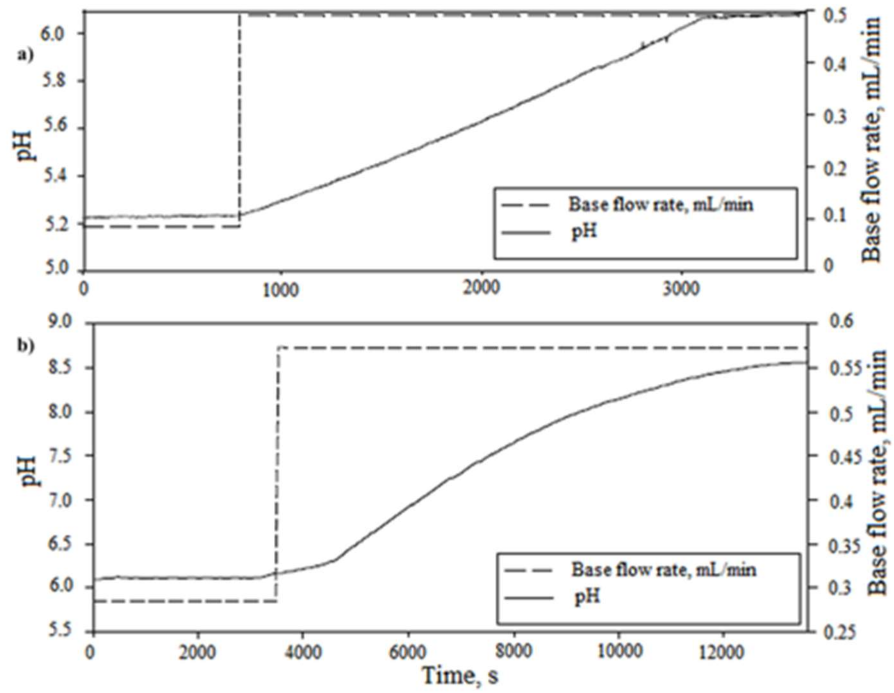

Figure 3. Response of the system to base flow rate-implementing consecutive positive step inputs a) bioreactor with growth medium and without microorganism was operated at steady state with constant acid flow rate of 0.081 mL/min (for the growth simulation) where step input given to base flow rate from 0.087 mL/min to 0.4581 mL/min and b) bioreactor with microorganism was operated at steady state where step input given to base flow rate from 0.285 mL/min to 0.5737 mL/min during the exponential phase of the growth.

However, an accurate model could not be established in terms of dead time and time constant using the FOPDT model parameters derived from these methods, as they lacked consistency. As a result, further investigation was conducted using the advanced ARMAX model and RLS to calculate the model parameters from the experimental data. By performing a more detailed analysis of the resulting process models, it became possible to identify the most accurate FOPDT model.

System Identification for Determination of ARMAX Model Parameters

During the fermentation of *C. acetobutylicum*, square wave, random, and ternary inputs were applied to adjust the flow rate of 0.3 M NaOH. The ARMAX model parameters were then calculated using RLS, based on the observed pH fluctuations over time. The 2 L working volume bioreactor was set to an agitation speed of 600 rpm and a temperature of 37 °C.

The RLS method was used to estimate the model parameters a_1, a_2, b_0, and b_1 from the square, random, and ternary inputs. The FOPDT model was first identified by graphical analysis of the reaction curves in Figure 4. The average fitted parameters for the *case without microorganism* were Kp = 0.54, τ = 2.37 min, and θ = 0.52 min, with a coefficient of determination (R^2 = 0.93). For the *case with microorganism*, Kp = 0.41, τ = 3.22 min, and θ = 0.74 min were obtained (R^2 = 0.91). These results confirm a slower and more damped dynamic behavior in the presence of active microbial metabolism. To improve model fidelity, a second-order ARMAX model was subsequently identified using the same input-output datasets. RLS (forgetting factor=0.96, initial covariance=1000) was applied to iteratively estimate the parameters. The ARMAX structure significantly improved the predictive capability, achieving an average fitting accuracy of R^2 = 0.98 and a mean squared error reduction of 27% compared with the FOPDT model.

In using ternary and random sequences of input, successive experimental runs were conducted to identify systems. Initially, the ternary input was used to generate stepwise disturbances at discrete intervals in the base flow rate to help with the identification of various process parameters such as steady state gain, time delay, and time constant. After proper stabilization and recovery of the constants in the fermentation medium, random input signals were applied during a second run to challenge the model's robustness under unstructured and dynamically varying patterns of excitation. The experiments were conducted in this sequential manner to prevent overlap of signal effects and to have each dataset report a different and independent dynamic response suitable for successful model identification.

The system was first exposed to a square wave input for research purposes. Positive step inputs were then applied, and the flow rate of 0.3 M NaOH was elevated from 0.097 mL/min to 1.3 mL/min while the system remained in its second steady state (Figure 4(a)). Subsequently, a negative input was introduced to bring the flow rate back to its initial value of 0.097 mL/min, at which point the system had reached a second steady state (Figure 4(a)). The next phase involved the introduction of random inputs at the base flow rate to the system. The system was then trained on the following inputs after it had settled down at 0.367 mL/min: 0.85 mL/min, 0.1875 mL/min, and 0.5737 mL/min (Figure 4(b)).

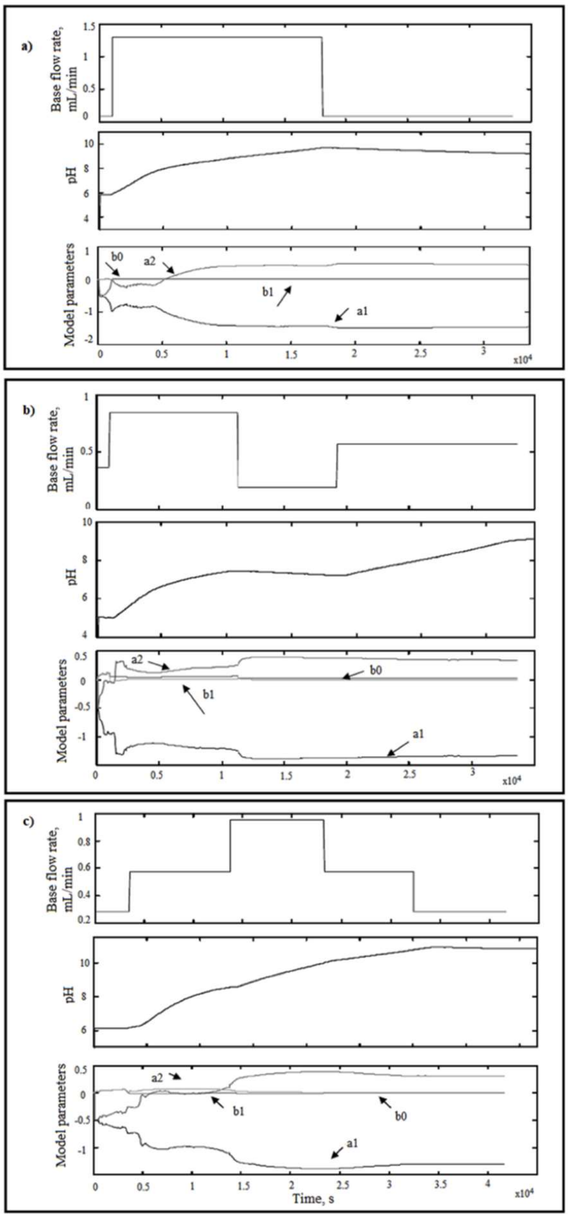

Figure 4. Experimental system identification results in biohydrogen production through dark fermentation. a) Square wave input, b) Random inputs, and c) Ternary inputs.

The last interruption was in the form of a ternary input composed of equal time intervals but with different amplitudes, which was fed to the system at 0.285 mL/min, 0.57 mL/min, and 0.95 mL/min (Figure 4(c)). All the flow rates used during the system identification test were selected based on the systematic step-change experimental design, which was to determine the dynamic pH control process based on different conditions, such as 0.097, 1.3, 0.367, 0.85, 0.1875, 0.5737, 0.285, 0.57, and 0.95 mL/min. Low flow rates were used to establish slow pH gradients and to examine the steady-state response and process lag characteristics, while high flow rates were used to induce fast transient dynamics and to determine the system sensitivity to larger disturbances. These were chosen from preliminary open-loop experiments to introduce disturban-ces within a safe and manageable pH range. Use of both small and large input variations included a guarantee that the discovered ARMAX and FOPDT models were accurate for the whole operating range and captured the nonlinear characteristics of the system.

Unlike the FOPDT model parameters, the ARMAX model parameters demonstrated similar behavior (Figure (4)). The model parameter values were relatively consistent with each other (Table S2). The ARMAX model parameters based on different excitation types (random, square, and ternary inputs) have also proved to be extremely regular in magnitude and sign, as shown in Table S2. Among them, the random excitation-trained model possessed the most consistent parameters and minimum fitting of the integral of squared error (ISE = 47.27), which confirms that random excitation provided the highest information content for system identification. Such consistency is a marker that the model could well represent the process dynamics irrespective of various operating conditions. The presence of the positive and negative coefficients within finite numerical ranges describes the inherent behavior of the bioreactor's pH dynamics, with the positive coefficients reflecting the immediate effect of the addition of bases on the increase in pH, and the negative coefficients the delay or compensatory effect resulting from buffering interactions and the delayed response of the liquid phase. The stability of these coefficients across several identification experiments confirms the stability of the ARMAX model and demonstrates that it was capable of accurately capturing the intrinsic input-output dynamics of the fermentation process under different excitation conditions.

From this perspective, since the model parameters derived through RLS with varying amplitudes and time intervals are nearly identical, it can be confidently stated that the ARMAX model is suitable for use in a pH control algorithm. To assess the performance, the ISE was calculated, with the random input-output data yielding the best estimation performance (ISE value of 47.27. The resulting process model is shown in Eq. 9 below.

$$y(t) - 1.1837\, y(t-1) + 0.1860\, y(t-2) = \\ = 0.044\, u(t-1) - 0.0011\, u(t-2) + e(t) \qquad (9)$$

where $y(t)$ is the output variable, $u(t)$ is the input variable, and $e(t)$ is white noise.

Although no independent verification experiments were performed, the validity of the identified models was confirmed through cross-validation with varying input signal types. For this purpose, model parameters that were identified with one type of excitation pattern (e.g., input signal square wave) were employed to predict the system response for a different type of excitation (e.g., random signal input). The resemblance between the pH responses simulated and experimentally measured validated the suitability of the FOPDT and ARMAX models for process dynamics description under various excitation conditions. The cross-validation procedure ensured the stability of predictive performance and reliability of the models without additional experimental measurements, as is typical in system identification research in process control problems.

The model used in this study, ARMAX, was identified using offline experimental data, but its structure and recursive estimation algorithm are inherently well-suited for eventual application in online environments.

Model parameters were estimated through Recursive Least Squares (RLS), which inherently supports iterative updating with further input-output data. This is an aspect that is the foundation of real-time or adaptive applications in continuous bioprocess monitoring. In long-term fermentations, however, because there are process nonlinearities, microbial growth fluctuations, and measurement noise, the quality of estimation can be compromised. To combat such issues, alternative methods such as adaptive filtering, variable forgetting factors for RLS, or hybrid ARMAX-neural network modeling could be applied. The expansions would provide disturbance robustness and dynamic tracking of time-varying system parameters, which would otherwise improve the practicability of the proposed approach in industrial fermentation control.

Determination of Theoretical Control Parameters of PID

The controller parameters, where K_C represents the proportional gain, τ_I the integral time constant, and τ_D the derivative time constant, were determined by means of the Cohen-Coon Method [40]. The Cohen-Coon Method applies the FOPDT model parameters to determine the controller settings. For this research, the second process reaction curve values, which were derived from three different model parameter estimation methods—transient analysis, Smith, and linear regression—were employed to compute the PID parameters (Table 1). Instead of using simulated acid flow rate values, the second process reaction curve data were chosen for the Cohen-Coon method, as it allowed the use of real experimental conditions with microorganisms, which expedited the whole process.

In the earlier research, controller performance was approximated primarily in terms of set point tracking performance employing ISE as the quantitative performance metric. In this manner, a good initial indication of control accuracy and dynamic response has been achieved. Although robustness measures such as gain margin and phase margin were not included in the present analysis, they are essential metrics for assessing whether a controller can maintain stable operation under process disturbances and uncertainties. Such frequency-domain analysis would be particularly relevant to large-scale or continuous fermentation systems, where microbial activity variations and process parameter variations can affect system stability. Future work will include gain and phase margin tests and disturbance rejection tests to more generally test the robustness and industrial viability of the control strategy designed.

The identified models were used to design and test three different PID tuning strategies: transient analysis, Smith method, and linear regression-based parameter estimation. Figure 5 illustrates the performance of the controllers relative to setpoint tracking and the overall error built up.

The transient analysis-based controller exhibited the shortest settling time (approximately 6 minutes) with minimum overshoot (< 3%) and the lowest ISE value of 50.82, justifying its superior overall control performance.

The Smith method yielded an ISE of 71.45 with small oscillations due to uncertainty sensitivity in time delay, and linear regression-based tuning provided an ISE of 63.59 with moderate response rate but low stability.

These results indicate that a controller's performance depends heavily on how well the dynamic model is. The reliance of the transient analysis technique on experimentally derived reaction curves made more accurate modeling of true process lag possible, leading to more stable and accurate pH control.

The best-fitting model was determined based on the model's performance and control settings (Figure 5). The "error" term in Figure 5 represents the ISE, a measure of the overall difference between actual and setpoint pH response throughout the control simulation. The ISE has a straight-forward representation of control performance by punishing larger deviations more than minor ones since it represents the ability of the system to track stable and exact pH levels. Lower ISE values indicate improved control accuracy and reduced oscillation. This metric was used to compare and assess the performance of different PID parameter tuning strategies and allow for an objective assessment of the accuracy and stability of the controller.

PID parameters were tested using a theoretical PID algorithm coded in MATLAB, employing a suitable second-order ARMAX model derived from random inputs during the system identification phase (Eq. 9), where a_1 = -1.1837, a_2 = 0.1860, b_0 = 0.044, and b_1 = -0.0011. Eq. (9) is the dynamic equation between the manipulated variable (base flow rate) and controlled variable (pH). The equation includes gain and delay dynamics derived from experimental data and is the foundation of control evaluation theory.

Table 1. Determination of PID control parameters by Cohen-Coon Method.

Calculation Methods of FOPDT Model Parameters	With microorganism			PID parameters			ISE
	K_p	θ	τ	K_c	τ_I	τ_D	
Transient Analyze	8.318	520	6300	1.995	1258.1	189.4	50.82
Smith	8.318	4590	3330	0.146	7952.1	1345.8	1.92×10^3
Linear Regression	8.318	3072	3280	0.202	5804	965.08	1.18×10^3

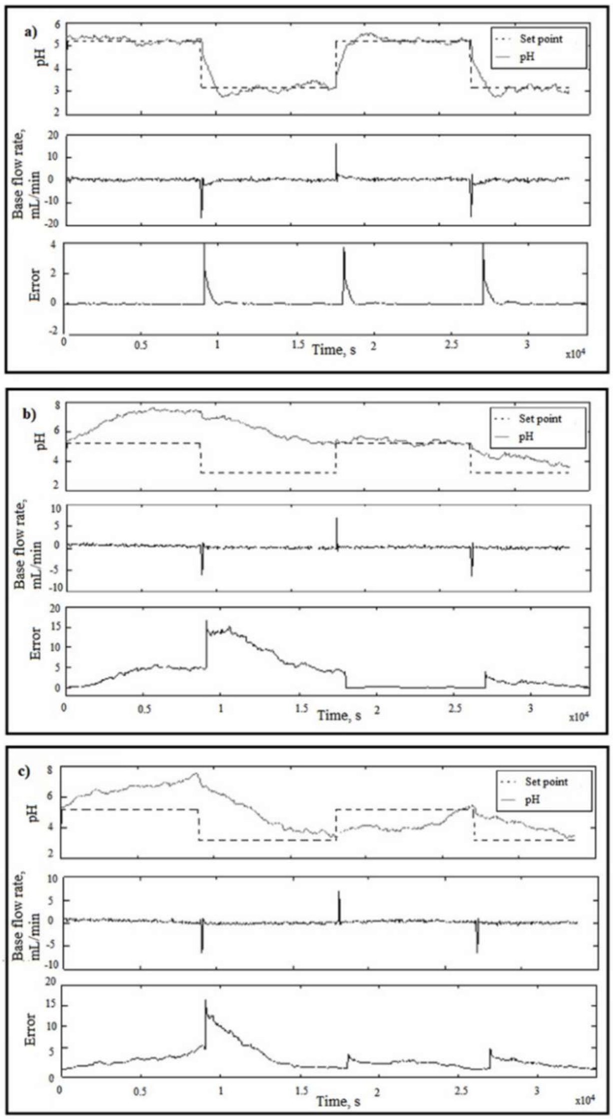

Figure 5. Theoretical PID control results with the suitable ARMAX model obtained from system identification where a_1 = -1.1837, a_2 = 0.1860, b_0=0.044, and b_1= -0.0011 with PID parameters obtained from Cohen-Coon as, a) Kc = 1.995, τ_I = 1258.1, and τ_D = 189.4, b) Kc = 0.146, τ_I = 7952.1, and τ_D = 1345.8, and c) Kc = 0.202, τ_I = 5804, andτ_D = 965.

Use of this equation in simulation led to a realistic assessment of the control performance under different process conditions, confirming that the structure of the model identified can properly reflect the dynamic pH behavior of the fermentation process.

Error traces of Figure 5 are correlated with the ISE performance measure, reflecting cumulative deviation from setpoint to actual pH. Lower ISE derived from transient analysis tuning indicates better disturbance rejection and higher tracking accuracy compared to other methods.

The PID control with the lowest ISE value highlighted the impact of control parameters and model structure on performance. The most successful setpoint trajectories were achieved with the lowest ISE value of 50.82 based on the PID parameters from the transient analysis model, as shown in Figure 5(a) and Table 1.

A comparative summary of the established parameters and control performance results is presented in Table 1. Two salient points are made through the data: First, biological activity heavily modifies process dynamics, both lengthening time constant and delay; and second, model-based controller tuning based on experimentally observed dynamics produces considerably improved theoretical control results.

In general, the results validate that experimental system identification provides a reliable foundation for pH control design in fermentation processes. The outcome justifies using ARMAX modeling for adaptive control generation and the use of FOPDT-based transient analysis for initial practical tuning. Among the different PID tuning techniques tried, the optimum Integral of Squared Error (ISE) value of 50.8206 was obtained with the values obtained using the transient analysis method. This result confirms that the transient analysis-based tuning approach produced the best and most stable control performance with quick setpoint response and low overshooting and oscillation. This increased performance illustrates its application feasibility for biological process control, wherein slow response dynamics and system nonlinearities limit the applicability of conventional linear tuning approaches. Though the FOPDT model provides an easy first-order approximation of the bioreactor dynamics and enables straightforward PID tuning, it is fundamentally limited in its capacity to capture the nonlinear and time-varying characteristics of fermentation systems with accuracy. The FOPDT model parameters (Kp, τ, and θ) are assumed to be constant, while in fact they change with the variation of microbial activity, substrate consumption, and metabolic phase transitions. Thus, the FOPDT model can capture the system dynamics in only a limited operating range. Controller performance with FOPDT-derived parameters can deteriorate under exposure to process disturbances or microbial kinetic drift. In contrast, the ARMAX model—by incorporating autoregressive and moving average terms alongside the exogenous input—provides a more adaptive and responsive model structure to describe the true fermentation dynamics. This modeling strategy enhances the predictability and robustness of the control system, particularly during non-stationary process conditions typical of biological operations.

During recent years, biohydrogen production through dark and photo fermentation methods has made remarkable progress by continuously using dynamic modeling, optimization, and control techniques, which have led to the augmentation of hydrogen production. Montecchio *et al.* [36] and Reyes *et al.* [37] made an advanced version of Anaerobic Digestion Model No. 1 (ADM1) by using a variable stoichiometry method combined with mass balancing. This method gave deeper insights into the kinetics and thermodynamics of hydrogen production and consumption through the isotopic or microbiological methods [36,37]. Monroy *et al.* [38] made a neural network (ANN) model for hydrogen production from photofermentation of immobilized bacteria [38]. Jamali *et al.* [39] invented a mathematical model for the thermal biohydrogen fermentation in fluidized bed reactors (FBRs) that produced 2.2 mol H_2/mol of glucose [39]. Zuniga *et al.* [40] demonstrated that higher glucose concentrations during dark fermentation result in a higher hydrogen generation rate [40]. Herein, dynamic modeling and system identification were performed, controlling pH and manipulating base flow rate. The FOPDT model and its parameters were applied to determine PID settings, and the finest second-order ARMAX parametric model was used to ascertain PID control parameters. The pH control system was validated by successful setpoint tracking. Previous research has considered several strategies for improving the control performance of bioprocesses through dynamic analysis and parameter optimization. However, most strategies rely heavily on simulation-based validation without direct incorporation of experimentally determined system dynamics. The integration of experimental data into model-based control development remains a relatively unexplored field in biochemical process engineering. The present research fills this gap by experimentally identifying the system dynamics of pH variation in *C. acetobutylicum* fermentation and subsequently applying the identified models to theoretical controller analysis. This methodology mix enables the development of a practical and experimentally grounded conceptual framework for future implementation of adaptive control in bioreactor systems.

CONCLUSIONS

A comprehensive dynamic analysis and system identification of pH was conducted using fermentation by *C. acetobutyricum* in a batch bioreactor to develop an appropriate equation. The findings of this study demonstrate that the dynamic behavior of pH variation in the fermenter system can be precisely predicted by the mathematical model presented in Eq. (9). The equation establishes the relationship between the rate of base addition and the ensuing pH response, with both gain and delay terms determined from the experimentally established system parameters. The accuracy of this model reconfirms its sufficiency as the right equation for performance evaluation and controller tuning. In creating this experimentally validated model, the study provides a strong theoretical foundation for the future implementation of adaptive and model-based control strategies in fermentation processes. PID control simulations were used to assess the model's effectiveness. The dynamic study showed that while the FOPDT model parameters, derived from transient analysis, were useful for determining PID controller parameters using the Cohen-Coon method, the Smith and linear regression techniques did not provide reliable results. Smith and linear regression techniques yielded less precise parameter estimates compared to the transient analysis technique, primarily due to the fact that they are susceptible to process noise and time delay variability in biological systems. The Smith method relies on a steady-state approximation that cannot fully characterize the time-varying dynamics of fermentation processes, and the linear regression method assumes linearity and constant delay, which might not always hold in the case of dynamic microbial conditions. The transient analysis approach, however, provides parameter estimates directly from the experimentally determined reaction curve and thus can more accurately represent the true dynamic response. Its stability against variations in measurement and its ability to capture both slow and rapid process transitions are responsible for the great consistency of results obtained using this method. The system behavior was represented much more accurately by a second-order ARMAX model with the help of advanced inputs. Moreover, setpoint trajectories were also successfully realized, while the PID control parameters gave rise to the minimum ISE value of 50.82. Thus, it can be said that theoretical control studies, computer simulations, and modeling are nowadays less expensive and can still improve the fermentative yields. The approach taken in this paper would be suitable for any bacterial fermentation process to ascertain controller parameters from a simple model, then carry out control actions with an advanced model to keep the output at the desired level. This kind of methodology is a great hint for bioreactors compatible with PID that are already in use during industrial operations. The strategy developed in this work can be applied to other bacterial fermentation systems, but slight adjustments may be necessary based on microbial kinetics and process conditions. Micro-organism fermentations whose metabolic rates or substrate usage patterns differ here would possibly require respecification of the process parameters, such as gain, time constant, and delay time. In substrate-inhibited or nonlinear metabolic feedback systems, hybrid modeling techniques that combine ARMAX structures and nonlinear corrector modules, such as neural network or fuzzy logic models, would enhance the accuracy of predictions. In mixed-culture fermentations, where interspecies interactions create coupling effects, the control regime has to evolve into a multi-input-multi-output mode. Despite these challenges, the current methodology provides a good basis for developing flexible, data-driven control strategies for many different bioprocesses.

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NAUČNI RAD

KONTROLA U BIOREAKTORU KROZ IDENTIFIKACIJU EKSPERIMENTALNOG SISTEMA I DINAMIČKU ANALIZU

Kiseli nusproizvodi proizvedeni tokom fermentacije mogu izazvati pad pH vrednosti, što zauzvrat utiče na rast mikroorganizama i formiranje proizvoda. Da bi se pH vrednost održala na željenom nivou, neophodna je kontrola procesa. Cilj ove studije je razvoj prediktivnog modela za ponašanje pH vrednosti tokom fermentacije Clostridium acetobutylicum kroz dinamičku analizu i identifikaciju sistema. Model prvog reda plus mrtvo vreme (FOPDT) i model autoregresivnog pokretnog proseka drugog reda sa egzogenim (ARMAX) bila su dva pristupa koja su upoređena. Dok je FOPDT model korišćen za izvođenje parametara PID regulatora putem tranzijentne analize, Smitove i linearne regresione metode, ARMAX model, identifikovan metodom rekurzivnih najmanjih kvadrata, izabran je zbog svoje bolje tačnosti u snimanju dinamike ulaza i izlaza. Podešavanje PID-a je obavljeno Koen-Kun metodom. Rezultati simulacije su pokazali da je praćenje zadatih vrednosti uspešno izvršeno i da je ARMAX model pružio tačniji prikaz sistema. Optimizovani PID kontroler je zabeležio minimalnu vrednost Integrala kvadratne greške od 50,82. Ova studija ukazuje na efikasne strategije modelovanja i kontrole za proizvodnju stabilnog pH tokom fermentacije, pružajući tako veoma korisno znanje za druge bioprocese koji zahtevaju preciznu kontrolu.

Ključne reči: dinamička analiza, identifikacija sistema, FOPDT model, ARMAX model, teorijska PID kontrola.