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OPTIMIZED MEDIA DESIGN AND FEEDING STRATEGIES IN MAMMALIAN FED-BATCH CULTURES USING MODEL PREDICTIVE CONTROL AND MOVING HORIZON ESTIMATION

TESIS PARA OPTAR AL GRADO DE MAGÍSTER EN CIENCIAS DE LA INGENIERÍA, MENCIÓN QUÍMICA

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RESUMEN DE LA TESIS PARA OPTAR AL GRADO DE
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POR: FRANCISCA KATHERINE PIZARRO GALLEGUILLOS
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DISEÑO OPTIMIZADO DE MEDIOS Y ESTRATEGIAS DE ALIMENTACIÓN EN CULTIVOS FED-BATCH DE MAMÍFEROS UTILIZANDO CONTROL PREDICTIVO POR MODELO Y ESTIMACIÓN DE HORIZONTE MÓVIL

Las cultivos de células de mamíferos son esenciales para la producción de proteínas terapéuticas complejas debido a su compatibilidad con las modificaciones postraduccionales humanas. Mejorar la proliferación celular y el rendimiento de biomoléculas es crucial para aumentar la eficiencia de producción y la rentabilidad de bioproductos. A pesar de los altos rendimientos logrados a través de la investigación sobre la expresión génica, el metabolismo y el crecimiento, las estrategias actuales suelen ser empíricas, costosas y lentas. Los avances en la composición del medio de cultivo y el control procesos han impulsado mejoras en la productividad. Para elevar aún más los niveles de producción, estos avances deben combinarse con estrategias de alimentación y medios de cultivo optimizados, además de monitoreo y control en tiempo real para asegurar una suplementación precisa y óptima.

Este trabajo presenta un enfoque integrativo para optimizar medios de cultivo y estrategias de alimentación en cultivos fed-batch. Usando un modelo metabólico dinámico con parámetros optimizables, es posible predecir los requisitos nutricionales específicos de una línea celular en un proceso de producción particular. Esto permite diseñar medios y estrategias de alimentación personalizadas a cada sistema de producción. El diseño optimizado se logra mediante control predictivo por modelo (MPC), que ajusta la alimentación según las necesidades de las células para mantenimiento, crecimiento y generación de producto. Adicionalmente, se integran Estimación de Horizonte Móvil (MHE) y MPC para manejar la disponibilidad de datos y la variabilidad del proceso.

La implementación de MPC junto con MHE mantiene las variables controladas dentro de los rangos deseados y aumenta la concentración de biomasa en comparación con las estrategias tradicionales de control en lazo abierto y proporcional (P). Utilizando una alimentación continua, tanto el control P como NMPC logran métricas de rendimiento similares, mostrando iguales índices de desempeño ITAE, IAE e ISE. Mediante alimentación por pulsos, el NMPC supera al control P considerando restricciones operacionales y mejorarando los índices de ITAE, IAE e ISE, reduciéndolos en un 8.30%, 3.43% y 0.24%, respectivamente. En presencia de variabilidad del proceso, el esquema NMPC-MHE resultó en una disminución del 4.68% en el índice ITAE y un aumento del 5.34% en el IVCD en comparación con la estrategia en lazo abierto, mostrando mayor crecimiento de biomasa y una suplementación de nutrientes más eficiente. Los principales desafíos incluyen tiempos de muestreo rígidos y dependencia de mediciones precisas. El trabajo futuro debe centrarse en integrar variables fácilmente medibles para mejorar el modelo, facilitar la estimación de estados y reducir la necesidad de mediciones complejas. Además, incorporar esquemas de control robustos y mediciones con tiempos de muestreo variables podría mejorar el rendimiento de la estrategia de control.

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Mammalian cell cultures are essential for producing complex therapeutic proteins due to their compatibility with human post-translational modifications. Enhancing cell proliferation and biomolecule yield is crucial for improving production efficiency and cost-effectiveness. Despite high yields achieved through research on gene expression, metabolism, and growth, current strategies are often empirical, expensive, and time-consuming. Advances in culture media composition and process control have driven productivity improvements. To further enhance biomass and product yield; these advancements must be complemented by designing optimized feeding strategies, culture media, and real-time monitoring and control to ensure precise and optimal supplementation.

This work presents an integrative approach for optimizing culture media and feeding strategies for fed-batch cultures using a predictive mathematical model. The specific nutritional requirements of a given cell line in a particular production process are predicted by integrating cell and product composition with characteristic process parameters into a detailed dynamic metabolic model with optimizable parameters. This approach enables the design of customized media compositions and feeding strategies tailored to each production system. The optimized fed-batch design is achieved through model predictive control (MPC), where the feed is based on the cells' nutritional needs for maintenance, growth, and production. Furthermore, real-time monitoring and control challenges are addressed by integrating Moving Horizon Estimation (MHE) and MPC to manage limited data availability and inherent process variability.

Implementing MPC combined with MHE improves control accuracy, maintains controlled variables within desired ranges, and increases biomass concentration compared to traditional open-loop and proportional (P) control strategies. In continuous feeding scenarios, both P and NMPC control achieve similar performance metrics, with equal Integral of Time-weighted Absolute Error (ITAE), Integral of Absolute Error (IAE), and Integral of Squared Error (ISE) indices. In pulse-feeding strategies, NMPC outperforms P control by managing constraints and improving ITAE, IAE, and ISE indices, reduced by 8.30%, 3.43%, and 0.24%, respectively. In the presence of process variability, the NMPC-MHE control scheme resulted in a 4.68% decrease in the ITAE index and a 5.34% increase in the IVCD compared to the open-loop strategy, leading to better control, enhanced biomass growth, and more effective nutrient supplementation. Key challenges include rigid sampling times and heavy reliance on accurate measurements. Future work should focus on integrating easily measurable variables to improve the mathematical model, simplify state estimation, and reduce reliance on complex measurements. Additionally, incorporating robust control schemes and measurements with varying sampling times could enhance control strategy performance.

Como no hay tiempo,
siempre es el momento
de corregir
y de empezar

S. Larraín

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En primer lugar me gustaría agradecer profundamente a mi familia. A mis papás, Katherine e Iván, y hermano Benjamín que me han apoyado desde muy pequeña en todas las aventuras que me he propuesto. Sin ustedes nada de esto sería posible. Quién soy hoy y mis logros, no es gracias a mí, sino a todo el esfuerzo, tiempo y dedicación que pusieron en mi crianza. Gracias mamá por estar siempre conmigo. Sin duda alguna no me alcanzan los agradeciemientos para poder retribuir todo lo que me han dado.

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Chapter 1

Introduction

1.1. Motivation

Biopharmaceuticals are therapeutic products that are created by genetically modifying living cells or organisms. Some examples of biopharmaceuticals include proteins, peptides, nucleic acids, and other large molecules [1–3].

Mammalian cell cultures have become a widely used platform for obtaining bioproducts. These cells are the preferred hosts for the production of most complex therapeutic proteins, as their functional and pharmacokinetically relevant post-translational modifications are highly compatible with humans [1]. Therefore, to increase the production and cost-effectiveness of these products, it is necessary to increase the proliferation of producing cells and the yield of the biomolecule of interest per cell.

The fed-batch mode is widely utilized in industrial bioreactor operations for cultivating mammalian and microbial cells [4]. This method is particularly effective in cell cultures where achieving high cell density and overcoming common challenges such as substrate inhibition, catabolite repression, product inhibition, and glucose effects are required [5].

The high yields obtained in current processes are the result of years of research that has allowed us to better understand gene expression, metabolism, and growth [6]. However, these strategies are usually empirical, expensive, slow, and require plenty of experiments [7]. Overall, this increase in productivity is mainly due to improvements in culture media composition and process control [6–8].

The development process is usually iterative until the desired levels of growth and production are achieved on a large scale [1]. Commonly used strategies for media formulation and optimization present a series of limitations as they are mainly based on literature studies of related cell lines and extensive experimentation without considering the specific characteristics of the combination between host cell, clones, or product [1, 9, 10]. Among other less commonly used strategies are mass balances that allow estimating the minimum requirements of a culture based on stoichiometric relationships and cellular composition [11–14], statistically designed experimentation to obtain data that includes the interactions of the components without requiring exhaustive experiments (DoE) [9], and medium analysis to identify and enrich limiting nutrients [15]. Model-based strategies have been used less fre-

quently in the development of cell culture media due to an incomplete understanding of metabolic pathways and the vast number of medium components, in addition to their high complexity [1]. However, as biopharmaceuticals become more complex, the adoption of this approach is becoming increasingly popular to complement the task of media development since the use of models facilitates the generation of hypotheses and the preliminary testing of incipient ideas [16].

To ensure that these advancements in media formulation and optimization translate effectively into large-scale production, it is crucial to maintain stringent control over the bioprocess. The main aim of controlling a process is to ensure that it operates under the desired conditions while keeping safety and efficiency in mind and meeting environmental and product quality standards [17]. Fluctuations in process variables directly impact product quality, and the primary objective of process control is to mitigate these deviations. The most straightforward approach to achieve this is by maintaining rigorous control during the operation of the process, a strategy that has been preferred within the biopharmaceutical industry until the present [18].

Nevertheless, ensuring an effective and robust fed-batch operation can be difficult because of the necessity to sustain an optimal rate of substrate feeding. The concentration of substrate within the growth medium directly impacts the process affecting cell growth, and both desired products and by-product formation rates [19–22]. Consequently, maintaining an optimum substrate concentration is crucial for efficient nutrient utilization, reduction of unwanted by-products, and maximization of desired product yield [23].

Therefore, to enhance biomass and bioproduct production, it is crucial to implement a feeding strategy and culture media design that precisely defines the elemental needs of the cells in both batch and fed-batch culture conditions. This should be complemented with real-time monitoring and control to ensure adequate supplementation of the cultures.

1.2. Prior work context

Within the framework of the FONDEF ID18i10308 project: “Plataforma para el diseño de medios de cultivos: Optimización de la manufactura de bioproductos.” [24], a rapid, flexible, effective, and standardized tool was developed to define the essential needs of cells in culture, both under batch and fed-batch conditions, to adequately supplement the cultures and achieve higher productivity. This increase in proliferation and production, in turn, helps to reduce operational costs and waste production, thereby simplifying the purification of the final product.

The project is based on a dynamic model for the production of monoclonal antibodies in GS - NS0 cells [25]. This model is used to predict the requirements for amino acids and nutrients in animal cell cultures, and subsequently optimize the composition of the culture medium and the feeding strategy in fed-batch mode.

The proposed solution consists of several modules, one of which is the simulation module aimed at implementing the model described in [25]. For fed-batch reactors, the module allows simulation of the reactor operating in open-loop mode, i.e., with a manually defined temporal

trajectory for glucose and amino acid feed rates, or in closed-loop mode, where the feed rate trajectories are defined online by a feedback control system using a standard proportional (P) controller. The objective of the control system is to maintain glucose and glutamine levels at a desired value, defined as a set-point tracking problem. Since both nutrients play a key role in ensuring optimal cell growth and product assembly [19, 26, 27] monitoring and controlling them is essential for obtaining elevated product yields and cost-effectiveness of the process. The ultimate goal of this project is to provide an optimized feeding strategy and medium composition to be implemented in open-loop mode in the laboratory.

1.3. Problem Definition

Due to the significant fluctuations in process dynamics during operation, standard controllers with fixed parameters are insufficient for precise control [23]. This is mainly due to the nonlinear dynamics of the processes associated with exponential growth rates, metabolic changes, volume changes, and potential disturbances in the feeding process. In addition to changes in process dynamics, the effects of disturbances such as batch-to-batch variations in feed composition, initial biomass concentration, and noise in process measurements must also be considered [28]. The proposed strategies in the literature usually control a limited number of variables, neglecting the effect of the exhaustion of essential amino acids. Therefore, to complement the feeding strategy, a tailored media composition is obtained from the simulation of the specific consumption rate of each amino acid, ensuring a more comprehensive approach to process control and optimization.

In this thesis, a mathematical model for a CHO-tPa cell line was developed in collaboration with the Process Modeling and Distributed Computing Laboratory and Mammalian Cell Culture Lab. This model was employed to design optimal feeding strategies and enable real-time control of fed-batch reactors. By determining a media composition tailored to the nutritional needs of the culture, a model predictive control strategy provides optimized feeding curves, ensuring precise supplementation throughout the culture. The performance of this advanced control strategy was rigorously evaluated and compared to the existing P controller used on the platform, demonstrating its effectiveness and potential to enhance overall process efficiency. These dynamic consumption rates also consider particular characteristics of the cell lines used, such as cell composition, product composition, and the nutritional requirements of the specific clone.

The novelty of this work lies in its integrative approach to optimizing culture media and feeding strategy design for fed-batch cultures using a predictive mathematical model for control and real-time monitoring. This approach allows for incorporating specific parameters for each cell line, clone, or product. The primary goal of this strategy is to minimize experimental costs, time, and resource use through well-planned feeding designs and media compositions supported by extensive in-silico testing. Additionally, this approach accelerates process development and enables flexibility and customization, making it applicable to various cell lines and production scenarios.

1.4. Thesis Outilne

The thesis is organized as follows: Chapter 2 introduces basic concepts of process control that will be used throughout the text. Chapter 3 presents a case study, detailing the problem formulated by Pizarro et al. in a forthcoming publication. Finally, Chapter 4 summarizes the key findings of this work and guides future research in the field of bioprocess control.

Chapter 2

Process Control for Fed-batch Cultures

In the early 1900s, fed-batch cultures were used in yeast production from malt wort. Workers recognized that an elevated malt concentration in the medium could lead to alcohol formation, so it had to be maintained low enough to maximize the yield [29].

Since then, fed-batch has become a benchmark for bioprocesses, and it has become a common choice for production systems for several decades due to its robustness and elevated product yields [23]. Currently, most biotherapeutics in the market are produced using fed-batch fermentations [30] and it is the preferred strategy for large-scale production [31]. In large-scale mammalian cell culture, various bioreactors are employed. Large-scale culture typically refers to volumes greater than 100 liters of culture medium, which can reach capacities of up to 10.000 liters [32].

Bioprocesses are shaped by the mix of physical, chemical, and biological factors in the environment and the biochemical processes within microorganisms. Process control is crucial for ensuring system stability. Even a simple control loop can improve system performance when faced with disturbances. Therefore, these techniques are extensively applied in controlling biotechnological processes [33].

In this chapter fundamental aspects of process control are introduced. The purpose of this chapter is to introduce the basic concepts of process control to familiarize the reader with the terminology and concepts used in the case study presented in Chapter 3. Secondly, the main strategies based on feed rate manipulation in fed-batch cultures are presented and discussed.

2.1. Basic Control Definitions

Control is defined as a series of operations aimed at monitoring the state of a system, determining necessary control actions based on deviations from its desired state, and implementing these actions to minimize or eliminate these deviations [33].

A system comprises components working together to accomplish a specific goal. Any system being analyzed should have clearly defined conceptual boundaries but may interact

with external systems. A schematic process block diagram is depicted in Figure 2.1.a.

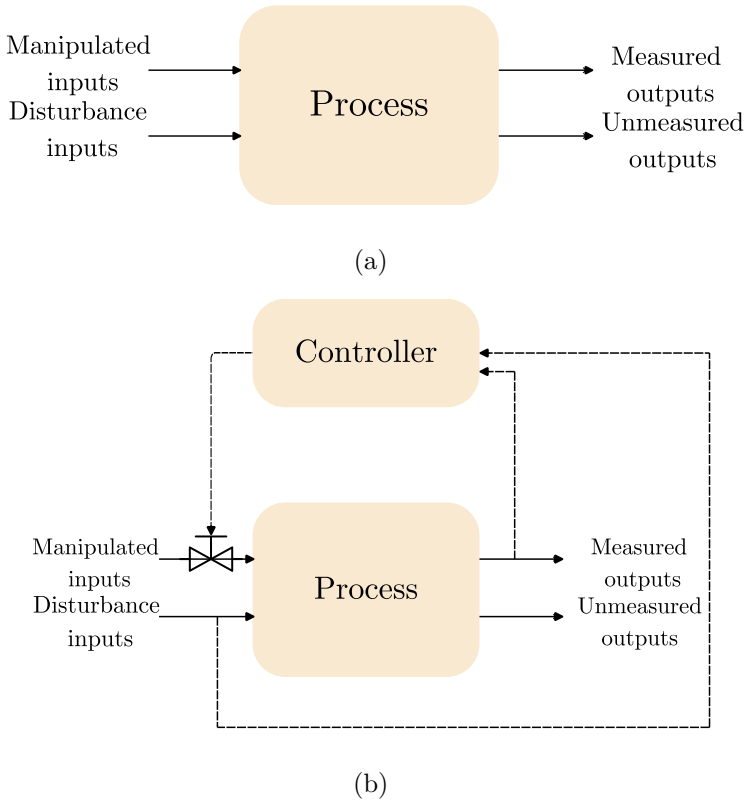


Figure 2.1: Conceptual Process Block Diagram. Diagram adapted from [34]. (a) Input-Output representation of a system. (b) Schematic process block Representation

Inputs are categorized as either disturbances or controllable inputs. Disturbances are values fixed by an external system that cannot be altered, while inputs can be adjusted as needed. Outputs are classified as measured or unmeasured. Utilizing measurements of process outputs or disturbance inputs is crucial for making informed decisions regarding the appropriate values of manipulated inputs. This is the function of the controller illustrated in Figure 2.1.b.

The design of a control system according to [34] involves identifying the following:

1. Control objective(s)
2. Input variables
3. Output variables
4. Constraints
5. Operating characteristics
6. Safety, environmental, and economic considerations

7. Control structure

The above steps will be described in the later sections.

2.1.1. Control Objectives

The objective of a control system can be separated into 3 main strategies:

1. Maintain the system output(s) at a desired value that can either be constant or variable over time, suppressing the effect of external disturbances.
2. Stabilize the process.
3. Optimize the process performance in terms of yields, productivity or profitability

Specifically, some examples of possible control objectives based on the performance of fed-batch processes, according to Mears et al [35]. include:

- Maximise product concentration
- Minimise by-product formation
- Maximise process yield or productivity
- Maximise biomass concentration
- Maintain an oxygen concentration profile

In this type of process, the challenge is not only to maintain the optimal feed rate but to identify how the optimal feed rate is defined. Since both overfeeding and underfeeding nutrients are detrimental to cell growth and product formation, developing a suitable feeding strategy is critical in fed-batch cultures [36]. In addition, there are multiple effects of the feed rate in the process, such as changes in substrate concentration, which can alter the specific growth rate and product formation rates. These effects are also observed because of variations in the volume caused by the feeding strategy adopted [35].

2.1.2. Input Variables and Output Variables

Input variables can be categorized as either manipulated or disturbance variables. Manipulated inputs can be adjusted by the control system or process operator, while disturbance inputs influence process outputs but cannot be altered by the control system [34]. A disturbance is a signal that tends to adversely affect the value of the output of the system [37]. Output variables can be classified as measured or unmeasured variables. Measurements may be made continuously or at discrete intervals of time, and inputs may vary continuously or at discrete time intervals. The controlled variable is a quantity or condition that is measured and controlled. Normally, the controlled variable is the output of the system [37]. Depending on the number of inputs and output systems can be categorized as:

- Single input-Single Output (SISO): For one control (output) variable, there exists one manipulated (input) variable that influences the process.
- Multiple input-Multiple Output (MIMO): Multiple control (output) variables are influenced by multiple manipulated (input) variables in a given process.

In fed-batch processes, the manipulated variable to be considered is the flow rate. There is a challenge in defining the controlled variable due to the lack of online sensors for variables directly applicable to feed rate, such as substrate concentration [35].

2.1.3. Constraints

All processes have operating constraints, which are classified as hard or soft [34]. An example of a hard constraint is the maximum allowable volume limit of a reactor —it specifies the maximum volume the reactor can hold without risking damage or compromising safety. An example of a soft constraint is a product composition or yield —it's preferable to define a composition within specific ranges or achieve a desired yield to ensure process efficiency, but deviation from these specifications may occur without presenting safety or environmental risks.

2.1.4. Operating Characteristics

Operating modes are classified as batch, continuous or fed-batch. Batch processes generally operate for a short period, and the operating conditions may vary during that time [34]. In a batch reactor, all necessary medium components are added at the beginning of the operation. Therefore, their concentrations are not controlled but can vary as the living cells grow. Basic controls for pH, temperature, dissolved oxygen, and foam are applied during batch culture [38]. In bioprocess engineering, batch culture is frequently used for the production of viruses for vaccines and gene therapy applications as well as in cell production for immunotherapy and other therapies [39].

Continuous processes run for long periods under relatively stable conditions before they are shut down [34]. In a continuous reactor, one or more feed streams containing the necessary nutrients are continuously added. At the same time, an effluent stream containing cells, products, and residuals is continuously removed [38]. Typically, this process operates in a steady state, maintaining a constant volume. Although common in the chemical industry, it is not prevalent in the bioprocessing industry due to challenges in maintaining sterility and because steady-state operations often produce inferior results compared to dynamic operations [38]. In continuous culture, the flow rate must be carefully controlled to avoid washing out cells faster than they can grow. Simultaneously, growth inhibitors and waste metabolites produced by the cells need to be removed to prevent growth inhibition. Balancing these requirements often results in low cell and product concentrations, making the process economically unfeasible [39]. Unlike chemical processes that remain stable over time, cells can mutate or undergo epigenetic changes. This can lead to variations in the cell population, productivity, and product quality.

A typical semi-batch or fed-batch process initially charges the reactor, with additional feed components introduced during the batch run. The simplest form of fed-batch culture involves intermittent harvest. At a late exponential growth stage of the culture, a portion of the cells and product are harvested, and the culture is replenished with fresh medium containing the nutrients required for cell growth and product formation. This avoids metabolite inhibition of cell growth and replenishes nutrients for continued cell growth [39]. Carbon sources, nitrogen, phosphates, nutrients, precursors, or inducers are intermittently or continuously added to the culture, adjusting feed rates dynamically without an effluent stream [38]. This manipulation

allows the concentrations of limiting nutrients to be maintained at a constant level or to follow a predetermined optimal profile.

2.2. Process Models and Dynamic Behavior

According to the *McGraw-Hill Dictionary of Scientific and Technical Terms* [40], a model is defined as:

> "A mathematical or physical system, obeying certain specified conditions, whose behavior is used to understand a physical, biological, or social system to which it is analogous in some way."

In the context of control-system design, we specifically refer to **mathematical models**. A process model, as defined by [34], is:

> "A set of equations (including the necessary input data to solve the equations) that allows us to predict the behavior of a chemical process."

Models are crucial in control-system design for several reasons:

1. **Simulation**: They allow us to simulate and predict the behavior of a process with a proposed control system, helping to anticipate and evaluate its performance.
2. **Embedded Control**: Models can be integrated into controllers to anticipate the effects of control actions, enabling more informed and effective adjustments.

Process models provide essential insights and predictions that guide the design and implementation of control systems, enhancing their efficiency and effectiveness.

2.3. Control Structure

2.3.1. Control System Models

Control systems can be broadly categorized into two fundamental types: open-loop and closed-loop control systems. Each type has distinct characteristics and applications based on how it manages the relationship between inputs and outputs.

Open-loop Control

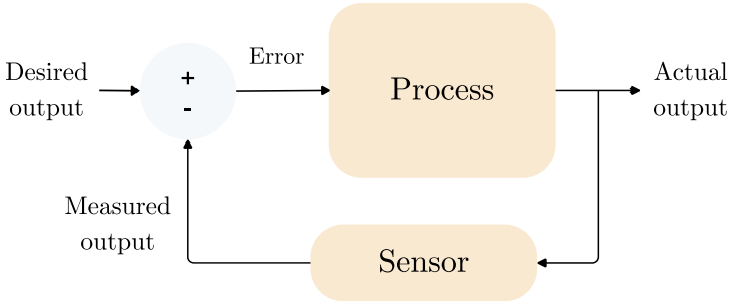
In an open-loop control system, the output has no effect on the input signal to the process. The output is determined solely by the initial setting or a predefined fixed sequence of inputs [41]. Open-loop systems have the advantage of being relatively simple and consequently cheap with generally good reliability, particularly if the process adheres to stable trajectories and the predictive model is sufficiently accurate [42]. But this is based on having an accurate process model, which never exists in practice due to modeling errors and system disturbances [37]. They can be inaccurate since there is no correction for errors in the output which might result from external disturbances. This is illustrated in Fig. 2.2.a.

Closed-loop Control

In control systems, the concept of "feedback" is crucial. Without feedback, the system operates solely based on the input signal [37]. In a closed-loop control system, the system's output is "fed back" to the input and compared with the desired value. The resulting difference, or error, between the actual and desired values is used to adjust the system's output, ensuring it maintains the desired performance [41]. This is illustrated in Fig. 2.2.b. In feedback control, no corrective action is taken until after the disturbance has upset the process, that is, until after the controlled variable deviates from the set point.

The two standard control types are feedforward and feedback. A feedforward controller measures the disturbance variable and sends this value to a controller, which adjusts the manipulated variable. The important advantage of feed-forward control is that corrective action is taken before the controlled variable deviates from the set point [34]. A feedback control system measures the output variable, compares that value to the desired output value, and uses this information to adjust the manipulated variable [34].

(a) Open-loop representation of a control system



(b) Control Representation

Figure 2.2: Eschematic representation of basic control system models

Illustrative Example

To introduce the essential components of a control system an illustrative example is going to be addressed. A simple process is illustrated in Fig 2.3. In this example, a fluid stream F_1 is fed to a tank, and an effluent stream F_2 exits the tank downstream.

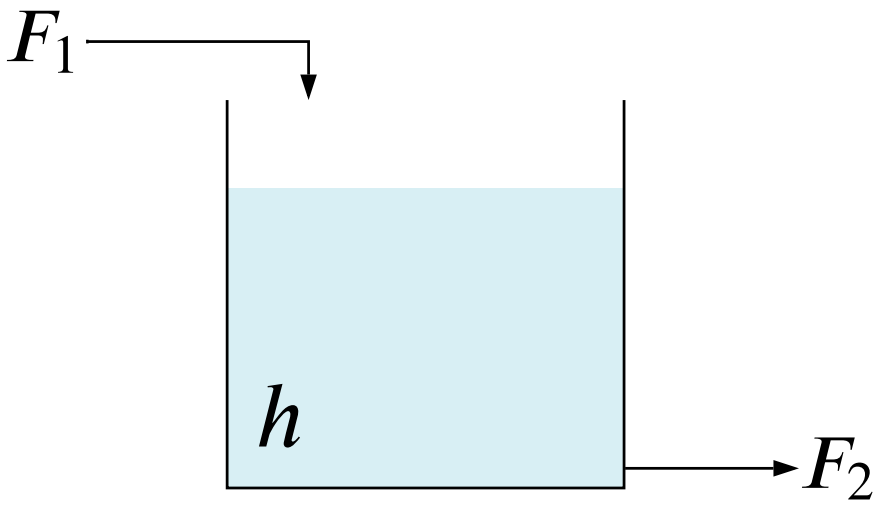


Figure 2.3: Surge Tank Problem: Process Flow Diagram

The control objective is to maintain the height h of the liquid inside the reactor within certain bounds. More specifically, a fixed height h_{sp} is going to be selected, which is going to be the setpoint. The input variables are the flows F_1 and F_2 and the output (controlled) variable is the height h, which we assume is a measured variable. We also are going to assume that there is a continuous inlet F_1 and outlet F_2 flow so that the process is continuous too. The inlet F_1 is determined upstream, leaving F_2 to be manipulated and defined by the controller.

Closed-loop feedback control

In this case, the measured output is the height. Since F_1 is an external input to the system and not a controlled variable, it acts as a disturbance. The control system must compensate for changes in the inlet, adjusting F_2 to maintain the controlled variable at its setpoint. The piping and instrumentation diagram (P&ID) for the feedback case is shown in Fig. 2.4. The level transmitter (LT) sends the measured height of liquid in the tank (h_m) to the level controller (LC). The LC compares the measured level with the desired level (setpoint) h_{sp} and sends a pressure signal (P_v) to the valve changing the flow rate F_2 through the valve. The difference between the setpoint and the measured process output h_m is also known as the error e.

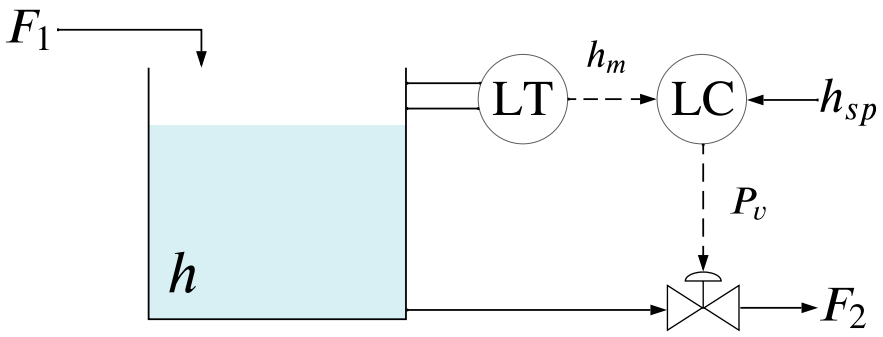


Figure 2.4: Surge Tank Problem: Process and instrumentation diagram for the feedback control case. Dashed lines indicate signals between different pieces of instrumentation. Figure adapted from [34]

This can be represented as a process control block diagram, shown in Fig. 2.5, where each block corresponds to a dynamic element within the loop. The diagram illustrates the components of a closed-loop control system, clearly showing why it is referred to as a "closed-loop". Three essential components are considered in the loop: the sensor or measurement device (level transmitter) that measures the height of the liquid in the tank, the actuator (valve) which changes the flow rate, and the controller, which determines the appropriate variation in the actuator's position (valve position). The process block itself relates the manipulated input to the process output (see Fig. 2.2.a).

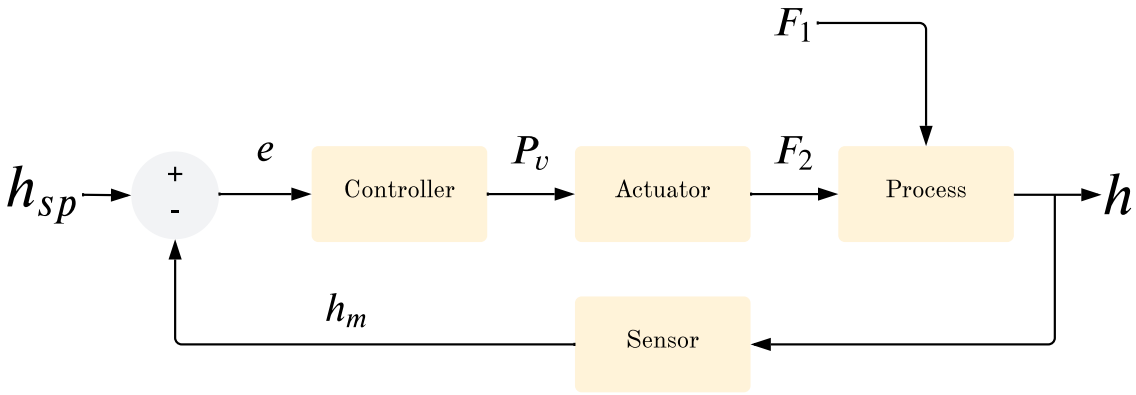


Figure 2.5: Surge Tank Problem: Block diagram for the feedback control case. Figure adapted from [34]

Control block diagrams are used to analyze the dynamic effect of feedback control loops. These diagrams visually represent the system components and their interconnections, showing how input signals are processed to produce output signals. All dynamic elements in a control loop are combined, usually using their Laplace transfer function representation [34]. A transfer function model characterizes the dynamic relationship of two process variables, a dependent variable (or output variable) and an independent variable (or input variable) [43].

Open-loop control

In this scenario, control actions are independent of the system's actual outputs. The manipulated variable F_2 is manually set or pre-programmed based on anticipated conditions, with no real-time adjustments made in response to the actual liquid height in the tank. Consequently, the system cannot compensate for disturbances or deviations, as there is no feedback mechanism to adjust F_2 according to the measured output, potentially leading to either overflow or underfilling of the tank, depending on the magnitude of the disturbance. This lack of real-time correction makes open-loop control less effective in maintaining consistent process performance compared to a closed-loop system. The block diagram representing this scenario is shown in Fig. 2.6.

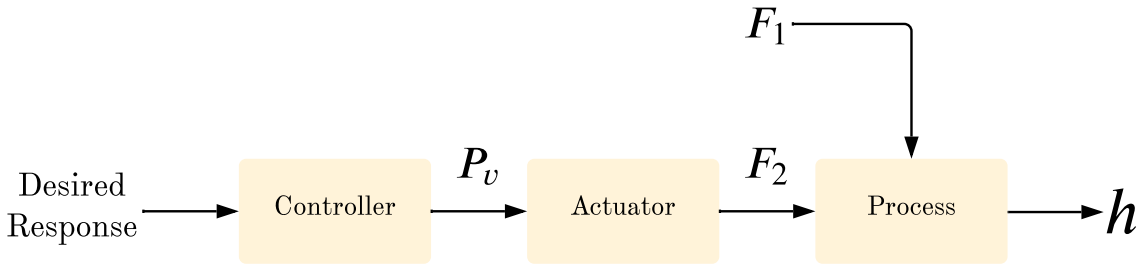


Figure 2.6: Surge Tank Problem: Block diagram for the feedback control case.

2.3.2. Control Algorithms

Once the control structure is determined, selecting the appropriate control algorithm becomes crucial. The control algorithm, sometimes referred to as the control law, uses measured output variable values to adjust the manipulated input variable. This can be achieved through an explicit control law, such as PID control, or more advanced methods like model predictive control, neural network control, or fuzzy logic control. These strategies will be described and discussed in the next section.

2.4. Review of Different Control Strategies

The creation of a robust control strategy depends on the understanding of the process and the availability of accurate process models. There is a direct correlation between the level of process understanding and the robustness of the control system. Low process understanding paired with inadequate process instrumentation is likely to fail, while elevated process understanding coupled with an elaborate monitoring system can lead to robust and consistent processes [44]. Depending on the specific requirements, an optimal choice can be made between using sophisticated instrumentation with complex control laws or simpler control laws paired with detailed monitoring systems [44].

2.4.1. Open-loop Control

Open-loop control is employed to apply a pre-calculated feeding profile to the process, based on the initial states and the operating conditions of the culture [35]. Feed rate profiles can also be calculated based on a process model, often referred to as model-based control. This method requires a robust process model, and its effectiveness is heavily dependent on the

accuracy of the model itself [23]. Examples of open-loop control strategies have been used for increasing batch-to-batch reproducibility of bioprocesses, by controlling the specific growth rate μ during the biomass growth phase to achieve improved process control.

Jenzsch *et al.* [45] propose an open-loop control strategy for recombinant protein production in *E. coli*, where instead of targeting the maximum growth rate, $\mu_{\max}$, they maintain the growth rate, μ, at a lower setpoint, μ_{set}, through controlled substrate feeding. This reduces batch-to-batch variability caused by differences in initial biomass concentration.

Similarly, Aehle *et al.* [42] addresses the issue of inconsistencies in final cell concentrations due to small variations in initial cell density. They suggest regulating the glutamine feed rate to keep the growth rate below the maximum, which stabilizes the process by allowing cells with excess substrate to grow faster until reaching the target concentration, while cells with limited substrate slow down their growth. While both approaches enhance process reproducibility, they come at the expense of a slightly extended cultivation time. In the specific case examined, operating at a lower specific growth rate increased the biomass formation phase by approximately 2 hours.

In Tebbani *et al.* [46] the authors' primary goal is to determine the optimal feed rate profile over time to maximize either biomass growth or the production of desired metabolites. Once the optimal feeding strategy is determined, the researchers propose a cascade control system to track this optimal trajectory, ensuring that the actual process closely follows the calculated ideal.

In open-loop control, there is no adaptation to disturbances, meaning any disturbances in the feed cannot be corrected. While this approach may not always result in the highest product yields and does not account for system disturbances, it can still be a valid method for feed rate control in certain bioprocesses. One of the key benefits of open-loop control is its simplicity of implementation, as it does not rely on measurements or feedback. However, this simplicity comes with significant limitations, including the need for pre-computed knowledge of profiles and the challenges associated with the mathematical formulation of non-linear systems [44].

2.4.2. Closed-loop Control

Proportional-Intregative-Derivative Control

The standard feedback control algorithms (also called control laws) are widely used in the process industries [44, 47]. This also applies to biotechnology processes [48]. Proportional-integral-derivative (PID) control and on-off control are the predominant types of feedback control [43].

In feedback control, the objective is to reduce the error signal $e(t)$ to zero where:

$$e(t) = y_{sp}(t) - y_m(t) \tag{2.1}$$

and y_{sp} corresponds to the setpoint and y_m the measured value of the controlled variable. Although the the set point can be time-varying, in many process control problems it is kept constant [43]. A diagram representing a feedback controller is shown in Fig. 2.7.

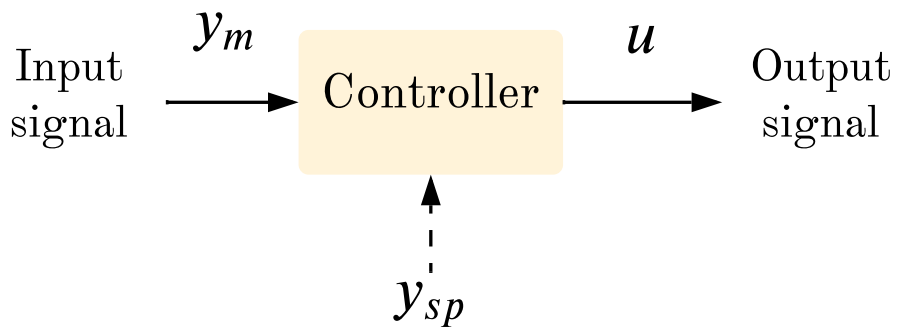


Figure 2.7: Diagram of a simple feedback controller. The output signal is denoted by $u(t)$, the input signal by $y_m(t)$ and the setpoint by y_{sp}. Figure adapted from [43].

In PID control the control signal is defined by three terms: the proportional term (P), the integrative term (I) and the derivative term (D):

- Proportional: For proportional control, the controller output ($u(t)$) is proportional to the error signal. The controller gain K_c can be adjusted to make the controller output changes as sensitive as desired to deviations between the set point and controlled variable.

$$u(t) = K_c \cdot e(t) \tag{2.2}$$

- Integrative: For integral control action, the controller output depends on the integral of the error signal over time. Where τ_I is an adjustable parameter referred to as the integral time.

$$u(t) = \frac{1}{\tau_I} \int_0^t e(t)dt \tag{2.3}$$

  Integral control action is widely used because it provides an important practical advantage, the elimination of offset. Thus, when integral action is used, u changes until it attains the value required to make the error zero [43].

- Derivative: The function of derivative control action is to anticipate the future behavior of the error signal by considering its rate of change [43]. Then, the controller output is proportional to the rate of change of the error signal.

$$u(t) = \tau_D \frac{de(t)}{dt} \tag{2.4}$$

Finally, the control signal can be calculated depending on the type of control strategy employed. For Proportional-Integral-Derivative (PID) control, the control signal is given by:

$$u(t) = K_c \cdot e(t) + \frac{1}{\tau_I} \int_0^t e(\tau)d\tau + \tau_D \frac{de(t)}{dt} \tag{2.5}$$

where $u(t)$ is the control signal, K_c is the proportional gain, τ_I is the integral time constant, τ_D is the derivative time constant, and $e(t)$ is the error signal. In this case, the control law combines proportional, integral, and derivative actions. Closed-loop strategies can also be adaptive, where the control system automatically adjusts the controller parameters to

compensate for changing process conditions [34]. In fed-batch processes, PID control is usually implemented in the form of indirect feedback control schemes that couple the substrate feed rate with measurements of pH or dissolved oxygen concentration [23]

Other applications of PID control are discussed by Zhang *et al.*, where a method for controlling methanol concentration during fermentation processes using *P. pastoris* yeast was developed. The authors implemented a closed-loop control system based on a PID (proportional, integral, derivative) controller, with parameters optimized using a Pichia growth model and frequency response analysis. This approach proved superior to the conventional "on-off" control strategy, which often struggled to maintain stable methanol concentrations.

In related work by Hizbullah *et al.* [49], various control schemes for managing the glucose feed rate during fed-batch baker's yeast fermentation were evaluated. The study compared fixed-gain proportional-integral (PI), scheduled-gain PI, adaptive neural network, and hybrid neural network PI controllers. The controllers used the difference between the specific carbon dioxide evolution rate and oxygen uptake rate as the control variable. The evaluation focused on setpoint tracking and disturbance rejection. Results indicated that conventional controllers performed unsatisfactorily, with significant oscillation and offsets.

Finally in a work by Kager *et al.* [50] MPC was applied to a *Penicillium chrysogenum* fed-batch process and compared with PI(D) and MBC controllers. The MPC, which utilized a particle filter and a simplified kinetic model for state estimation, outperformed PI(D) and MBC by preventing by-product formation and ensuring efficient substrate utilization, despite the challenges posed by nonlinear process dynamics. Experimental verification showed that nonlinear process dynamics caused unstable PI(D) behavior.

Despite its optimal performance in linear processes, the use of PID-based control in nonlinear processes is limited, mainly due to the lack of reliable online or express at-line measurement systems for determining control parameters and the inherent nonlinearity of biological processes [51, 52]. Although widely used in the bioprocess industry due to their ease of implementation [53–55], ensuring good performance critically depends on the correct configuration and tuning of controller parameters to address process variability [48].

Artifical Neural Network based Control

An Artificial Neural Network (ANN) is a data-driven modeling technique that can describe a complex non-linear system without the need for explicit model equations. The method is classified as supervised learning and, therefore, receives a set of input data, such as observable quantities like pH or substrate composition, and returns predictions of a specified quantity, such as biomass concentration, referred to as outputs [56].

The structure of an Artificial Neural Network (ANN) does not have any physical meaning, despite its origins in modeling neuron interactions. ANNs are composed of layers of nodes, often called artificial neurons. These nodes are arranged into specific layers: an input layer, one or more hidden layers, and an output layer, as shown in Fig. 2.8. Each node in the network is interconnected with others through weighted connections, and it also has a threshold value. When the node's output exceeds this threshold, the node becomes activated and transmits data to the next layer in the network. The transformation from an input

vector to an output vector is achieved through a series of linear combinations, specified by the weights, and nonlinear operations, determined by an activation function [56].

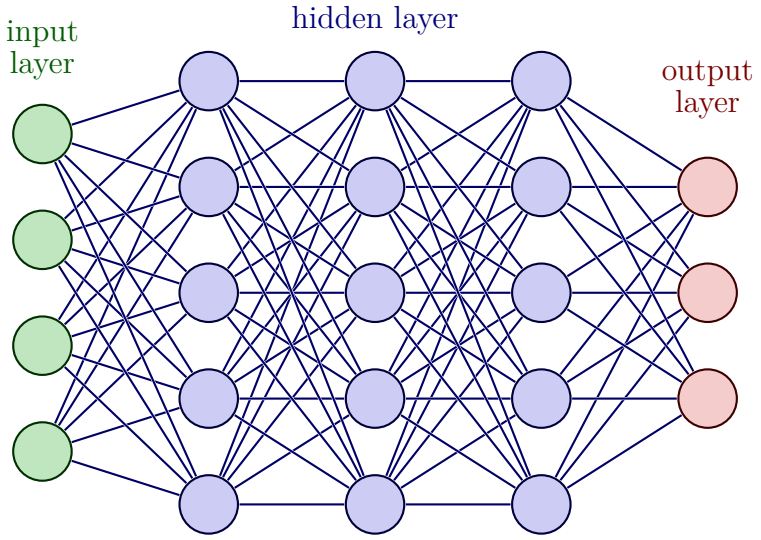


Figure 2.8: Neural network diagram. The circles represent interconnected neurons.

Artificial Neural Networks (ANNs) have become a valuable tool in bioprocess engineering, with diverse applications in modeling, optimization, and control. They are commonly used for online prediction of key process variables or integrated directly into optimization algorithms to determine optimal control strategies [57, 58].

Chaudhuri *et al.* [59] employed a feedforward neural network (FNN) model, trained on experimental data, to predict the nonlinear relationships between substrate feed rate and bioreactor outputs like cell mass and product concentration, according to the authors eliminating the need for a detailed kinetic model. They then use the trained FNN model to optimize substrate feed rate profiles. The effectiveness of this approach is demonstrated through examples of secreted protein and invertase production, where the FNN model successfully predicts bioreactor dynamics and generates optimal feed rate profiles that align closely with traditional methods.

Pantano *et al.* focused on designing a multivariable control system for a fed-batch bioprocess using neural networks for state estimation of unmeasurable variables. The neural networks are trained to estimate state variables over a range of operating conditions, accounting for perturbations and uncertainties to ensure robust performance. The performance of the controller proposed by Pantano et al. is compared against a PI controller. The study indicates superior performance of their proposed controller, especially in the presence of disturbances.

In the work presented by Zhang *et al.* [60] the authors present a hybrid modeling framework that integrates physics-based and data-driven modeling to enable online process monitoring, prediction, and optimization in an *in-silico* experiment. It utilizes a simple kinetic model to generate high-quality data from noisy measurements and handles missing data points, overcoming the limitations of data-driven models that require large, high-quality

datasets. The ANN, trained on refined data, predicts future process states (biomass, nitrate, and product concentrations) and enables the determination of optimal control actions throughout the process.

While ANNs have shown success in predicting biological system behavior and various control applications, they come with limitations, such as the need for large amounts of historical data and limited extrapolation capabilities beyond the training data [61]. These challenges can be mitigated by integrating ANNs with physics-based models, as this hybrid approach can compensate for the high data requirements of ANNs and extend their applicability.

Fuzzy Control

Fuzzy inference is a tool to incorporate linguistic rules into computational algorithms for application to process control. Fuzzy logic requires no initial knowledge of the dynamics of the system. Instead, the user's experience with the process is utilized to control the process based on an evaluation of the current state of the process [35]. Fuzzy control is based on the principles of fuzzy logic and is categorized into two types: the direct fuzzy control of process variables such as feed rate in fed-batch culture and temperature in batch operation, and the indirect control of bioprocesses in which the phase recognition is first done by fuzzy inference using process variables such as DO, glucose concentration, pH and so on and then the control strategies having been constructed in each phase are used for the process operation [62].

Fuzzy control utilizes fuzzy set theory, where variables can have degrees of membership to different sets. For instance, instead of defining a temperature as simply "high" or "low", fuzzy control allows for a range of possibilities like "slightly high", "moderately high", or "very high." This concept is represented by membership functions, which assign a degree of membership (between 0 and 1) to a specific value within the variable's range. This process is called fuzzification [62].

The fuzzy sets are then used to interpret the current state of the system. This control strategy relies heavily on capturing expert knowledge and translating it into a set of *IF-THEN* rules. These rules establish relationships between input variables (e.g., DO concentration, glucose level) and output variables (e.g., feed rate, temperature). This is how the user experience with the process is incorporated into the controller. For instance, a rule for controlling the glucose feed rate in a bioreactor might be: "*IF* concentration is *Big* and glucose concentration is *Small* and product concentration is *Small*, *THEN* change in feed rate is *Big*." This rule essentially states that if the DO is high (indicating good oxygen availability for growth) and both glucose and ethanol are low, the feed rate should be increased ("Big"). The collection of these rules forms the knowledge base of the fuzzy control system. The output of the rules is called defuzzification [63].

Numers *et al.* [64] developed a knowledge-based system utilizing fuzzy inference for supervisory control in bioprocesses, focusing on setpoint regulation and incorporating fuzzy logic principles. This approach aimed to enhance the control mechanisms in bioprocessing systems by leveraging fuzzy logic to handle the inherent uncertainties and complexities in biological systems.

Siimes *et al.* [65] also explored the application of real-time fuzzy-knowledge-based control

in Baker's yeast production, emphasizing the utilization of fuzzy logic and a knowledge base for fault diagnosis and control in bioprocesses. Their work highlighted the significance of incorporating real-time control strategies based on fuzzy logic to improve the efficiency and reliability of bioprocessing operations.

In summary, fuzzy control methods are valuable for managing nonlinear systems and are often more intuitive for users, as they rely on linguistic rules rather than complex mathematical models. However, their application in substrate feed rate control has become less common in recent years [23, 35]. The effectiveness of fuzzy logic controllers depends heavily on a thorough understanding of the bioprocess, as developing an accurate rule base requires substantial human expertise.

Model Predictive Control

The essence of Model Predictive Control (MPC) is to optimize the forecasts of process behavior over the manipulable inputs. This forecasting is achieved through a process model, making it an essential component of an MPC controller [66]. The control action is obtained by solving a finite-horizon optimal control problem at each sampling instant. The dynamic system is optimized over a finite prediction horizon, resulting in a finite control sequence, with the first control action in this sequence applied to the plant [67]. The prediction is evaluated based on the optimization of a cost function over the full process time. This may be to maximize the production or minimize the cost, or to follow a trajectory for a certain variable [68]. The optimization is also subject to predefined constraints which are built into the optimal control problem [68]. The simplified block diagram of the MPC control loop is shown in Fig. 2.9.

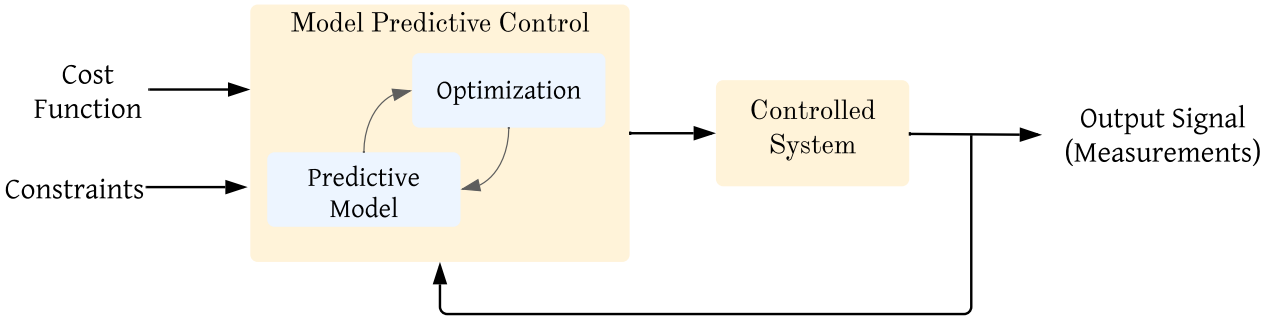


Figure 2.9: Simplified block diagram of an MPC-based control loop.

The process model can be either a classical mechanistic model, which typically describes cell growth and metabolism [69, 70], or an empirical model, such as those based on artificial neural networks [71, 72], partial least squares [73], and others. While empirical models can be highly accurate within the range of the process parameters from which they were derived, they often lack scalability and offer a less analytical perspective compared to mechanistic models [23]. Consequently, the effectiveness of the controller is closely tied to the quality of the predictive model used, with performance diminishing as operating conditions deviate from those under which the model was initially set [67].

One of the key reasons for the success of model predictive control is its capability to explicitly consider constraints and forecast the system's behavior [74]. Furthermore, it of-

fers intuitive parameterization by adjusting a process model, though it comes at a higher computational effort cost than classical controllers [75]. While widely applied in the process industry [75], MPC has found relatively few applications in bioprocess engineering, especially due to challenges in measuring critical quality attributes and robust measurements of key state variables [35, 76].

Various examples of MPC applications for the control of yeast cultures [71, 72, 77], bacteria [70, 78] and mammalian cells [69, 79, 80]. Some examples of their use are applied in alcohol biosynthesis [71, 72], recombinant proteins [80], hormones [77], antibiotic production [73] and maintaining cultures in a specific metabolic state [81]. However, half of these studies were carried out on various types of simulators and did not study the operation in real systems.

Kuprijanov *et al.* [70] demonstrated that an MPC controller can be implemented in industrial bioreactor automation systems for batch fermentation processes. Using only standard sensors (pH, OD) and online biomass and glucose measurements, and varying the substrate feed rate, the authors were able to demonstrate the ability of the MPC controller to follow a pre-set biomass growth profile and thus improve the repeatability and safety of the process [70].

Aehle *et al.* [80] used an MPC system to ensure reproducibility in an animal cell culture (CHO) for the production of a recombinant therapeutic protein. The control aimed to identify and control an optimal specific growth rate by controlling the rate of oxygen consumed by the cells by manipulating the glutamine supply. The authors estimated that the performance of the controller was quite good, given the high batch-to-batch reproducibility obtained in cultures operated with this controller.

MPC has some important limitations to consider. One key requirement for successfully implementing an MPC scheme is having a robust process model, which can be challenging if the system is not well characterized or if limited information is available. Additionally, the accuracy of the model needs to be validated with experimental data, which may require multiple runs [82]. Another limitation of MPC is its high computational cost, which can lead to significant execution times [44, 83]. It also has high hardware and software requirements, similar to those needed for artificial neural networks (ANNs) [82].

To conclude, model predictive control is a powerful closed-loop control method for substrate feed rate control, optimizing control actions across the entire process duration rather than just at the current time instant. MPC incorporates the impact of disturbances into the optimization problem, enhancing its robustness. However, the effectiveness of MPC heavily relies on the accuracy of the process model and its ability to manage unexpected disturbances. One of the significant drawbacks of MPC is the need for robust process models, which may not always be available, and its high computational expense, especially when optimizations are required at every time point [82]. While MPC is a standard method in other industries, its broader application in biological processes requires substantial advancements in process modeling. With robust models, MPC can offer a powerful and flexible control approach, capable of optimizing entire processes and addressing a wide range of control objectives.

2.5. Concluding Remarks

To define a proper control strategy, the process should begin with clearly defining the control problem, identifying the relevant input and output variables, and understanding their effects on the controlled variable. Next, it is crucial to consider the operating characteristics of the process and any physical constraints of the system.

The choice of the optimal control strategy depends on several factors. Understanding the process dynamics, as well as the availability of measurements and sophisticated equipment, is crucial in this decision. When measurement data is abundant, a simple control loop may be sufficient. However, in biological processes, where measurements are less frequent, approaches based on process understanding and mathematical models become more valuable. These model-based strategies not only help overcome the limitations of sparse data but also provide in-depth insights into the process.

When a process model is available, utilizing this knowledge to develop flexible control strategies, such as model-based control or Model Predictive Control, offers significant benefits. In particular, they provide the flexibility to tailor the objective function to meet the desired outcomes of the process. However, for broader industrial application of these model-based methods, continuous focus on process model development and uncertainty analysis is crucial to ensure that models are robust enough and applicable to control processes at an industrial scale. While PID-based control is commonly used in the bioprocess industry due to its simplicity, its effectiveness in non-linear processes is limited by the lack of reliable online measurement systems and the inherent complexities of biological systems.

In situations where developing a reliable first-principles model is challenging due to limited process understanding or system complexity, Artificial Neural Networks offer a valuable data-driven alternative. ANNs can learn complex relationships directly from historical data without requiring a deep understanding of the underlying biological mechanisms, making them particularly effective for modeling intricate and nonlinear relationships between process variables, such as substrate feed rate and product formation.

For an advanced control system to be effective in industrial applications, its performance must justify its implementation cost. Implementing advanced strategies, especially those requiring models, demands significant time and resources. Therefore, the cost should be minimized, and the method should not be overly complex to avoid increasing the cost through additional user intervention and man-hours. These factors should be carefully considered when evaluating new control strategies.

In conclusion, this chapter has outlined the basics of process control, including the key steps in defining and managing control problems. It covered traditional methods like PID control and advanced strategies such as Model Predictive Control and Artificial Neural Networks. While basic methods are easier to implement, they have limitations in handling complex, non-linear processes. Advanced strategies offer significant benefits but come with higher costs and complexity. Future advancements will require a balanced approach, combining process understanding with robust modeling and practical considerations to optimize control strategies effectively.

Chapter 3

Case Study: Advancing Biomanufacturing Excellence: Harnessing Model Predictive Control for Optimal Process Efficiency in Mammalian Cell Cultures

This chapter presents a publication in preparation in collaboration with the Process Modelling and Distributed Computing Lab and the Mammalian Cell Culture Lab. The experimental data were obtained by Ivan Paredes, and the mathematical model is based on the work of Kontoravdi et al [84] and later utilized by Quiroga Campano et al [25]. For the CHO cell line used in this study, the model was developed in collaboration with Ana Quiroga, Aarón Canales, Bastián Herrera, and the author of this thesis. The development of the control strategies and optimization of the culture media were carried out by the author of this thesis. The list of authors includes Aarón Canales, Ivan Paredes, Bastián Herrera, Ziomara Gerdtzen, J. Cristian Salgado Herrera, Ana Quiroga Campano, and the author of this thesis (in random order).

3.1. Introduction

According to reports, the biopharmaceutical market is valued at about $516.79 billion in 2024 and is expected to reach $761.80 billion by 2029. However, high-end manufacturing and burdensome regulatory requirements limit the market's growth. [85]. In response to these challenges, the US Food and Drug Administration published the Process Analytical Technology (PAT) [86]. This guidance aims to accelerate process development, promote efficient manufacturing, and ensure regulatory compliance, product safety, efficacy, and quality —all crucial to meeting the increasing demand for biomanufacture. A desired goal of the PAT framework is to design and develop well-understood processes that will consistently ensure a predefined quality at the end of manufacturing. Consequently, this transition guides the industry from a quality-by-testing paradigm to a quality-by-design (QbD) approach [87]. Implementing QbD involves measuring Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs). More specifically, one of the guiding principles of QbD is incorporating

robust control strategies and PAT tools to ensure consistent process performance and product quality through monitoring and controlling previously identified CPPs [88, 89].

Mammalian cell cultures have become a widely used platform for obtaining bioproducts [90]. These cells are the preferred hosts for producing most complex therapeutic proteins due to their ability to perform post-translational modifications that are pharmacodynamically and pharmacokinetically relevant and highly compatible with humans [1]. The high yields achieved with current processes result from many years of research that have led to a better understanding of gene expression, metabolism, and growth [90]. This increase in productivity is partly due to improvements in media composition and process control [7, 90], both key elements in process development [4].

To ensure that these advancements in media formulation and optimization translate effectively into large-scale production, it is crucial to maintain stringent control over the bioprocess. One effective way to achieve this is by closely monitoring key nutrient levels, such as glucose and glutamine, which are essential for optimizing nutrient supply and ensuring optimal cell growth and product assembly. Controlling nutrient levels is vital as they significantly impact cellular metabolism and productivity. Precise control of glucose and glutamine, the primary carbon sources in CHO cells [19], has been shown to enhance nutrient utilization efficiency and improve recombinant protein production [26, 27], with similar benefits observed in hybridoma and HEK cells [20, 91]. Thus, effective monitoring and control of nutrient levels are fundamental for achieving desired bioprocess outcomes while ensuring safety, efficiency, and adherence to environmental and product quality standards [17].

Following the launch of the Quality by Design (QbD) initiative, there has been notable progress in real-time monitoring, mathematical modeling, and control. The integration of Process Analytical Technology (PAT) with mathematical models for bioprocess control and design has been recognized as crucial for successful QbD implementation [92]. Model-based strategies support hypothesis generation and preliminary testing of new ideas [16], reduce development times, and enhance productivity [93]. Specifically, knowledge-driven models offer the highest level of accuracy during the early stages of pharmaceutical development, especially when experimental data are limited [94].

This progress can be observed in the increasing adoption of advanced control techniques. For example, Artificial Neural Network ANN-based control [60, 95], deep neural network-based control [96], and Model Predictive Control (MPC) [27, 50, 97–102] are becoming more common due to recent improvements in software tools, algorithms, and sensor technology [93]. Advanced strategies effectively manage the complexity of biological and highly non-linear systems. Both ANN and deep neural network control excel in predicting system behavior but require extensive data and face challenges with extrapolation beyond the training set [61]. In contrast, MPC can handle operational constraints explicitly and forecast system behavior but relies on a robust process model, which can be challenging to develop [74].

In comparison, traditional methods like open-loop control [42, 45, 46] and Proportional-Integral-Derivative (PID) control [49, 103–105] are widely used in industry for their simplicity [33, 35]. Open-loop control is straightforward to implement as it doesn't rely on measurements but requires pre-computed profiles and has limitations in managing disturbances and

the inherent variability of processes [44]. PID control, while more adaptable, struggles with non-linear processes and the intrinsic nonlinearity of biological systems [51, 52].

In complex non-linear systems, such as mammalian cell processes, Model Predictive Control has shown effectiveness in managing multiple variables and constraints [44]. MPC aligns well with the Process Analytical Technology (PAT) initiative, facilitating real-time monitoring and rapid adjustments to maintain process integrity, thus enhancing control and efficiency [76]. In the presence of measurement and model uncertainties, state estimators such as the Kalman Filters (KF) [106–108] as well as the Moving Horizon Estimator (MHE) [97, 99, 109, 110] are used with MPC improve the accuracy of predictions. Moving Horizon Estimation further complements MPC by providing superior state estimation, particularly in systems with nonlinear dynamics [111] and subject to constraints compared to the KF [112]. Integrating MPC with MHE enables precise state estimation and tracking, even when discrepancies exist between the process and the model, by leveraging historical data and real-time sensor information to enhance control performance [110].

In the literature, the proposed control strategies for manipulating the feed rate in fed-batch processes often focus on controlling a limited number of critical process parameters (CPPs), such as glucose and/or glutamine [98, 100, 108], or aim to maximize cell growth and productivity by adjusting a single input feed [95, 97]. However, these approaches typically overlook the exhaustion of other essential amino acids and fail to address nutritional requirements for product assembly and energy supply. Additionally, they often neglect important clone-specific parameters which are crucial for assessing critical quality attribute (CQA) sensitivity and understanding process responses to changes in CPPs [25].

In this work, we present an integrative approach for optimizing culture media and feeding strategies for fed-batch cultures using a predictive mathematical model. By integrating cell and product composition and characteristic process parameters into a detailed dynamic metabolic model with optimizable parameters, we can predict the specific nutritional requirements of a given cell line in a specific production process. This allows for the design of customized media compositions and feeding strategies tailored to each production system. The optimized fed-batch design is achieved using model predictive control, where the feed is based on the cells' nutritional needs for maintenance, growth, and production. Furthermore, the real-time monitoring and control problem is addressed by integrating MHE and MPC to manage limited data availability and inherent variability in the fed-batch process. This approach not only minimizes experimental costs, time, and resource use through extensive in-silico testing but also accelerates process development. It enables flexibility and customization, making it applicable to various cell lines and production scenarios. The effectiveness of the proposed control strategy is demonstrated by comparing the use of classic P control and MPC for fed-batch design, highlighting the advantages of the proposed method in optimizing culture conditions and improving overall process control and efficiency.

In continuous feeding scenarios, both P and NMPC control achieve similar performance metrics, with equal Integral of Time-weighted Absolute Error (ITAE), Integral of Absolute Error (IAE), and Integral of Squared Error (ISE) indices. In pulse-feeding strategies, NMPC outperforms P control by managing constraints and improving ITAE, IAE, and ISE indices, reduced by 8.30%, 3.43%, and 0.24%, respectively. The NMPC-MHE controller exhibited a

lower ITAE index, indicating better accuracy in controlling the process under various disturbance scenarios. In the presence of process variability, the NMPC-MHE control scheme resulted in a 4.68% decrease in the ITAE index and a 5.34% increase in the IVCD compared to the NMPC open-loop strategy. However, sampling time was found to have a significant impact on glutamine estimation; frequent sampling is beneficial for maintaining effective control and ensuring higher cell density. Although the control system can function with less frequent sampling, performance tends to decrease as the interval between samples increases.

3.2. Materials and Methods

Culture Conditions

Experiments were conducted on a 500 mL MiniBio bioreactor (Applikon Biotechnology, Ltd., Netherlands) with a 200 mL working volume at an initial 0.3×10^6 [cells/mL] concentration. t-PA producing CHO TF 70R cells were inoculated from an exponential growth phase culture in 100 mL spinner flasks at 37°C and 100 rpm stirring speed, in a 95% humidity incubator with 5% CO_2, in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 (Gibco, ME090283L1) medium supplemented with 5% FBS (Hyclone, SH30910.03), 200 nM Methotrexate (Sigma, M8407), 0.1 $[g/L]$ pluronic F68 (Sigma, P1300), antifoam C (5 ppm), and 10 $[mL/L]$ of penicillin-streptomycin, with a final concentration of glucose (Sigma, G5146) and glutamine 4 $[mM]$ (Sigma, G1517). The culture's pH, dissolved oxygen (DO), temperature, and agitation were controlled at constant values of 7.4, 35% of air saturation, 37°C, and 100 rpm, respectively. 1M NaOH and CO_2 gas were used to control pH.

Sampling and Sample Analysis

At the time of inoculation, a sample is taken at time zero, and the cell concentration and viability are measured using microscopy. The remaining volume is centrifuged, and the supernatant is removed and subsequently filtered. This volume is stored under refrigeration for later measurement of glucose, lactate, and ammonium using the Y15 biochemical analyzer and amino acid profiling via HPLC. Daily samples were taken from the culture for 120 hours.

3.3. Model Based Feeding Strategy

3.3.1. Mathematical Model

An unstructured model utilizing Monod kinetics was developed to describe cell growth and metabolism. This model relies on mass balances and stoichiometric reactions, incorporating common assumptions such as a well-mixed bioreactor and precise control of culture pH, temperature, and dissolved oxygen concentration. The development of the mathematical model was based on the analysis of experimental data in batch mode, identifying the main groups of nutrients influencing cell growth and death, as well as parameters associated with glucose and amino acid metabolism. For further details on the complete development of the model, including observability analysis, identifiability analysis, and calibration, refer to the work by Canales *et al.* [113].

The model consists of a set of 27 ordinary differential equations (ODEs) describing viable cells X_v, dead cells X_d, glucose GLC, glutamine GLN, lactate LAC, ammonia AMM, amino acids AA_i (where i denotes each amino acid) and the volume V. $AA_{in,i}$, GLN_{in}, and GLC_{in} represent the concentration of amino acids, glutamine, and glucose in their respective feeds, $F_{in_{aa}}$ and $F_{in_{glc}}$. The main equations of the model are expressed as:

$$\frac{dV}{dt} = F_{in_{AA}} + F_{in_{glc}} - F_{out} \tag{3.1a}$$

$$\frac{d(V\ X_v)}{dt} = (\mu - \mu_d)\, VX_v - X_v F_{out} \tag{3.1b}$$

$$\frac{d(V\ X_d)}{dt} = \mu_d VX_v - K_{lys} VX_d - X_d F_{out} \tag{3.1c}$$

$$\frac{d(V\ GLC)}{dt} = F_{in_{glc}} GLC_{in} - F_{out} GLC - Q_{glc} VX_v \tag{3.1d}$$

$$\frac{d(V\ GLN)}{dt} = F_{in_{AA}} GLN_{in} - F_{out} GLN - Q_{gln} VX_v - K_{d,gln} V \cdot GLN \tag{3.1e}$$

$$\frac{d(V\ LAC)}{dt} = F_{out} LAC + Q_{lac} VX_v \tag{3.1f}$$

$$\frac{d(V\ AMM)}{dt} = F_{out} AMM + Q_{amm} VX_v \tag{3.1g}$$

$$\frac{d(V\ AA_i)}{dt} = F_{in_{AA}} AA_{in,i} - F_{out} AA_i \pm Q_{AA_i} VX_v \tag{3.1h}$$

Where μ, μ_d, Q_{glc}, Q_{gln}, $K_{d,gln}$, K_{lys} Q_{LAC}, Q_{amm} and Q_{AA_i} are the specific growth rate, specific death rate, specific consumption rate of glucose, specific consumption rate of glutamine, glutamine degradation constant, specific cell lysis rate, specific lactate production rate, specific ammonia production rate and specific consumption or production rate for the rest of the amino acids, respectively. The model parameters were either determined experimentally by previous work from our group or estimated using a weighted least-squares objective function. The most relevant parameters are listed in Table 3.1. The detailed model description is supplied in Appendix A.

Table 3.1: List of model parameters, their descriptions, and units.

Parameter	Description	Unit
$\mu_{d,lacmax}$	Specific death rate from lactate toxicity[a]	[1/h]
$\mu_{d,max}$	Theoretical maximum death[a] rate	[1/h]
μ_{max}	Theoretical maximum growth rate[a]	[1/h]
$K_{d,gln}$	Glutamine degradation constant[a]	[mM]
$K_{d,lac}$	Monod saturation constant for death based on lactate toxicity[a]	[mM]
K_{glc}	Monod saturation constant for growth based on glucose metabolism[a]	[mM]
K_{gln}	Monod saturation constant for growth based on glutamine metabolism[a]	[mM]
$K_{i,lac}$	Monod constant for metabolic inhibition from lactate [a]	[mM]
$K_{lim,glc}$	Monod inhibition constant for glucose limitation[a]	[mM]
$M_{gly,glc}$	Maintenance coefficient of biomass from energy production during glycolysis from glucose[a]	[mM/Cells h]
$M_{k,AA}$	Maintenance coefficient of biomass from energy production during TCA from amino acids[a]	[mM/Cells h]
$M_{k,glc}$	Maintenance coefficient of biomass from energy from glucose[a]	[mM/Cells h]
n_{glc}	Glucose Hill Coefficient for cell growth[a]	
n_{gln}	Glutamine Hill Coefficient for cell growth[a]	
n_d	Glutamine Hill Coefficient for cell death[a]	
$R_{lac,ala}$	Lactate-alanine ratio produced from glucose consumption during glycolysis[a]	
$Y_{gly,glc}$	Yield of ammonia from glutamine[a]	[Cells/mM]
$Y_{k,AA}$	Yield of biomass from energy from amino acids[a]	[Cells/mM]
$Y_{k,glc}$	Yield of biomass from energy production during TCA from glucose[a]	[Cells/mM]
$Y_{amm,gln}$	Yield of ammonia from glutamine[a]	[mM/mM]
$Y_{x,glc}$	Yield on biomass from glucose[b]	[Cells/mM]
$Y_{x,gln}$	Yield on biomass from glutamine[b]	[Cells/mM]

[a] Fitted parameters.
[b] Experimentally determined parameters.

3.3.2. Optimized Media Composition

The medium composition was obtained from the simulation of the specific consumption rate of each amino acid for proliferation and energy production in batch mode. These dynamic consumption rates also consider particular characteristics of the cell lines used, such as cell composition, product composition, and the nutritional requirements of the specific clone. The composition of the growth medium is determined by fixing the concentration of glutamine GLN_{in} and calculating the ratio of the specific consumption rates of other amino acids ($\dot{q}_{AA_i}$) relative to $\dot{q}_{gln}$ the specific consumption of glutamine. This calculation follows the equation:

$$AA_{in,i} = \frac{\dot{q}_{AA,i}}{\dot{q}_{GLN}} \cdot GLN_{in} \tag{3.2}$$

Where $AA_{in,i}$ denotes the amino acid concentration in the optimized medium.

3.3.3. Control Objective

The objective of the control problem is to maintain glucose and glutamine levels at a desired set point, commonly defined as a set point tracking problem. For the closed-loop control problem, it is assumed that all state variables are measured. The controlled variables are the glucose and glutamine levels in the culture, and the manipulated variables are the glucose and glutamine feed rates. For the control problem, a reduced model is used, which includes the mass balances for glucose, glutamine, lactate, ammonia, viable cells, dead cells, and volume.

In the proposed strategy, all amino acids are considered implicitly, and the media design approach is used to ensure their availability, since cells cannot synthesize essential amino acids. The media composition links all amino acids to glutamine, which simplifies the model structure by allowing control over a single variable. Without this specific media design, the strategy would fail, as some amino acids would become depleted, leading to cell death due to starvation effects not accounted for in the mathematical model.

3.3.4. Bioreactor Operation

The feeding strategy—i.e., glucose and amino acids feeding rates—is determined using three different control strategies: Classic P control, nonlinear model predictive control (NMPC), and nonlinear model predictive control with moving horizon estimation (NMPC-MHE). The first two strategies assume no measurement noise or disturbances and are used to design the feeding strategy. The reactor operation was simulated in closed-loop mode to determine the size of the feeding pulses required to maintain glucose and glutamine levels around their set points. Once the strategy is determined, it is applied in open-loop mode, with no possibility of adjusting the control action. The third strategy simulates a real-time implementation, accounting for limited measurement availability and measurement noise. This approach addresses the challenges posed by the lack of measurements and inherent variability in the fed-batch process.

The feeding strategy is applied in pulses with an 8-hour interval rather than a continuous or steady flow, causing significant changes in the system's state trajectories. As a result, logical operations, known as switches, need to be integrated into the model [114]. A switch entails updating the state variables, leading to a discontinuous piece-wise dynamic model. For this, an event-driven method is used to identify discontinuities, and when the solution encounters an event, the solver updates the states and restarts from that point [115].

3.3.5. PID Control

In PID control, the goal is to minimize the error, which is the difference between the controlled variables and their setpoints, to zero. For proportional control, the controller output is directly proportional to the error signal Then, the manipulated variables or inputs $\mathbf{u}$ follow a simple control law defined by:

$$\mathbf{u} = F_{in} = K_c \cdot \mathbf{e}(t) \tag{3.3}$$

$$\begin{bmatrix} F_{in_{glc}} \\ F_{in_{gln}} \end{bmatrix} = \begin{bmatrix} K_{c_{glc}} & 0 \\ 0 & K_{c_{gln}} \end{bmatrix} \cdot \begin{bmatrix} x_{glc} - x_{sp_{glc}} \\ x_{gln} - x_{sp_{gln}} \end{bmatrix}. \tag{3.4}$$

Where $\mathbf{e}(t)$ corresponds to the error signal, which is the difference between the variable $\mathbf{x}$ at time t and its set point $\mathbf{x}_{sp}$. The key idea behind proportional control is that the controller gains K_c can be adjusted to make the controller output changes as sensitive as desired to deviations between set point and controlled variable [43]. The controller gains were tuned by optimizing the objective function presented in Eq. 3.5 using the Nelder-Mead simplex

method and, it considers the error of the controlled variables glucose and glutamine, weighed by the matrix $\mathbf{Q_P}$. An additional regularization term was added to find the optimal values of $\mathbf{K_c}$ weighed by the matrix $\mathbf{R_P}$.

$$\min_{Kc} \quad \int_{t_0}^{t_f} t\|\mathbf{x}(t) - x_{sp}\|^2_{\mathbf{Q_P}} + \|K_c\|^2_{\mathbf{R_P}} \tag{3.5}$$

However, we are only interested in minimizing the differences between glucose x_{glc} and glutamine x_{gln}, and their respective setpoints. Therefore, we can rewrite Eq. 3.5 as:

$$\min_{Kc} \quad \int_{t_0}^{t_f} t\left((x_{glc}(t) - x_{sp_{glc}})^2 q_{p_1} + (x_{gln}(t) - x_{sp_{gln}})^2 q_{p_2}\right) + \|K_c\|^2_{\mathbf{R_P}} \tag{3.6}$$

Where q_{p_1} and q_{p_2} are the diagonal elements of the weighting matrix $\mathbf{Q}_P$ corresponding to glucose and glutamine, respectively.

Despite PID controllers being the predominant choice in industrial settings and their widespread use in biotechnology due to their ease of implementation [44, 47, 48, 53–55], these methods encounter notable challenges. Their effectiveness relies on the accurate configuration and tuning of controller parameters to handle process variability [48]. Moreover, while PID controllers are well-suited for linear processes, their ability to handle the complexities of nonlinear processes is limited, leading to suboptimal performance in such scenarios [51, 52].

The control strategy using proportional control results in a continuous feeding strategy. To be suitable for the actual reactor operation, it must be adjusted to a pulse feeding strategy. For the pulse approximation, the integral of the glucose and glutamine feedings is calculated using the trapezoidal method at 8-hour intervals from the start of the feeding. The obtained volume for each step is then converted into a 2-minute pulse, and finally, the feeding strategy is applied to the system in an open-loop manner.

3.3.6. Model Predictive Control

The essence of Model Predictive Control (MPC) lies in optimizing the forecasts of process behavior based on controllable inputs. This forecasting relies on a process model, which is a fundamental component of an MPC controller [66]. In MPC, control actions are determined by solving a finite-horizon optimal control problem. The dynamic system is optimized over a finite prediction horizon, generating a sequence of control actions. The first action in this sequence is then implemented in the process [67]. While widely applied in the process industry [75], MPC and advanced control methods have only found relatively few applications in bioprocess engineering, especially due to challenges in measuring critical quality attributes and robust measurements of key state variables [35, 76]. In this regard, existing process analytical technologies (PATs), such as Raman spectroscopy and near-infrared spectroscopy, have been utilized to enhance real-time process control and monitoring [116]. However, these methods require highly sensitive and optimized instrumentation. Moreover, the deployment of these technologies demands significant investment and specialized expertise, which can be a barrier for many organizations [117]

We denote the vector of states $\mathbf{x} \in \mathbb{R}^{n_x}$, the input vector $\mathbf{u} \in \mathbb{R}^{n_u}$ and the vector of measured outputs $\mathbf{y} \in \mathbb{R}^{n_y}$. The differential equations of the mathematical model are denoted

as $f : \mathbb{R}^{n_x} \times \mathbb{R}^{n_u} \to \mathbb{R}^{n_x}$ and $g : \mathbb{R}^{n_x} \to \mathbb{R}^{n_y}$ as the measurement function. Then, the system in its continuous form can be described as follows:

$$\dot{\mathbf{x}}(t) = f(\mathbf{x}(t)), \quad t \neq \tau_k \tag{3.7a}$$
$$\mathbf{x}(t^+) = f(\mathbf{x}(t), \mathbf{u}(t)), \quad t = \tau_k \tag{3.7b}$$
$$\mathbf{y}(t) = g(\mathbf{x}(t)) \tag{3.7c}$$
$$\mathbf{x}(t_0) = \mathbf{x}_0 \tag{3.7d}$$

Where t_0 is the initial time of the culture with an initial condition of the reactor $\mathbf{x}(t_0)$, τ_k, $k \in \mathbb{N}$ corresponds to discrete-time events where a pulse is applied, and t^+ is the time instant after a pulse. For the feeding strategy design, we assume that all states are measured.

At each time, MPC calculates the optimal feeding pulses of glucose and glutamine by minimizing an objective function based on predictions of the system over a horizon T_p. The schematic representation of the MPC framework is shown in 3.1. The interval at which a measurement is taken, the control action is calculated, and the prediction is updated is called the sampling time T_s [74]. Similar to the proposed P controller, the objective of the MPC is to track a predefined fixed set point, i.e., to maintain glucose and glutamine concentrations around a certain reference level $\mathbf{x}_{sp}$. Two major constraints are considered in the optimization problem related to operational constraints. First, feeding can only occur at fixed intervals determined by τ_k. Second, if feeding occurs, the minimum and maximum flow rates defined by the controller cannot exceed those values allowed by the pump, F_{min} and F_{max}.

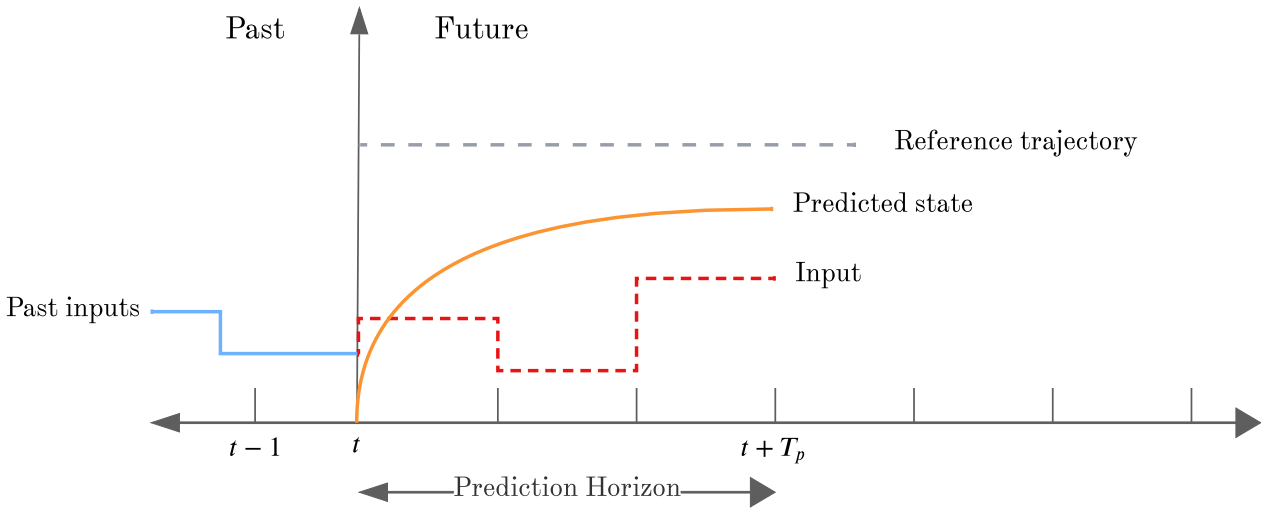


Figure 3.1: Schematic representation of the MPC framework. At each time step, MPC calculates the optimal control inputs by predicting the future behavior of the state variables over a prediction horizon.

The MPC problem is formulated within a finite time horizon T_p over which predictions are made:

$$\min_{\mathbf{u}(t)} \quad \int_t^{t+T_p} \|\mathbf{x}(t) - \mathbf{x}_{sp}\|_Q^2 + \|\mathbf{u}(t)\|_R^2 \tag{3.8a}$$

$$\text{s.t} \quad \dot{\mathbf{x}} = f\left(\mathbf{x}(t)\right), \quad t \neq \tau_k \tag{3.8b}$$

$$\mathbf{x}(t^+) = f\left(\mathbf{x}(t), \mathbf{u}(t)\right), \quad t = \tau_k \tag{3.8c}$$

$$\mathbf{y}(t) = g\left(\mathbf{x}(t)\right), \quad \forall t \tag{3.8d}$$

$$0 \leq \mathbf{x}(t) \leq \mathbf{x}_{max}, \quad \forall t \tag{3.8e}$$

$$F_{min} \leq \mathbf{u}(t) \leq F_{max}, \quad t = \tau_k \tag{3.8f}$$

$$\mathbf{x}(t_0) = \mathbf{x}_0 \tag{3.8g}$$

Where $\mathbf{u}(t)$ denotes the sequence of inputs that minimize the objective function. $\mathbf{Q}$ and $\mathbf{R}$ are the state weighting matrix and control weighting matrix, respectively. Similar to the (P) control problem, the diagonal elements of the weighting matrix $\mathbf{Q}$ correspond to the weights of glucose and glutamine, while the diagonal elements for the remaining state variables are set to zero. Note that to solve the MPC problem, the current state $\mathbf{x}_0$ needs to be known, which can be either measured or estimated. To solve the optimization problem, a direct multiple shooting method was used, with a fourth-order Runge-Kutta method for discretizing the differential equations. Direct methods convert the infinite-dimensional optimal control problem into a finite-dimensional nonlinear programming problem (NLP). In direct multiple shooting, control inputs are discretized into piecewise segments on a coarse time grid, and the ordinary differential equations (ODEs) are solved independently over each interval [118]. The NLP problem is solved using CasADi [119], an open-source tool for nonlinear optimization and algorithmic differentiation, and IPOPT [120], a software package for large-scale nonlinear optimization.

3.3.7. Moving Horizon Estimation

Moving horizon estimation (MHE) uses a model to forecast a system's behavior over a certain horizon based on an initial estimate of the system's state known, as prior, and then optimizes to find the smallest disturbances necessary to explain the system's measurements [121]. The current state of the system is inferred from a sequence of past measurements within a finite horizon N_e, which includes the most recent $N_e + 1$ measurements. When estimating, we assume that the measurements of the states are disturbed by Gaussian noise $\mathbf{v}$ and the model dynamics by state noise $\mathbf{w}$. Then, the MHE problem can be formulated as:

$$\min_{\mathbf{x}_k, \mathbf{v}_k, \mathbf{w}_k} \|\mathbf{x}_0 - \tilde{\mathbf{x}}_0\|_{\mathbf{P}}^2 + \sum_{k=0}^{k+N_e} \left(\|\mathbf{v}_k\|_{\mathbf{R}_v^{-1}}^2 + \|\mathbf{w}_k\|_{\mathbf{Q}_w^{-1}}^2 \right), \tag{3.9a}$$

$$\text{s.t.} \quad \mathbf{x}_{k+1} = F\left(\mathbf{x}_k, \mathbf{u}_k, \right) + \mathbf{w}_k, \tag{3.9b}$$

$$\mathbf{y}_k = h\left(\mathbf{x}_k, \mathbf{u}_k\right) + \mathbf{v}_k, \tag{3.9c}$$

$$0 \leq \mathbf{x}_k \quad \forall\ k = 0, \ldots, N_e \tag{3.9d}$$

Where $x_k = [x_0 \ldots u_{Ne}]$, $v_k = [u_0 \ldots u_{Ne-1}]$ and $w_k = [u_0 \ldots u_{Ne-1}]$ denote the sequences of states, measurement noise, and state noise estimates respectively, that are most likely to have produced the observed measurements and $F(\cdot)$ and $h(\cdot)$ are the discretized version of Eq. 3.7a and Eq. 3.7c, respectively. The first term on Eq. 3.9a represents the stage cost

where the a priori estimate of the initial condition for the horizon is given by $\tilde{x}_0$, while $\mathbf{P}$ is the weighting matrix reflecting the confidence in the initial condition [122]. The process noise vector w_k and measurement noise vector v_k are independent and generally assumed to follow a Gaussian distribution of zero mean with covariance matrices $\mathbf{Q}_w$ and $\mathbf{R}_v$, respectively [121]. By using the $\mathbf{Q}_w$ matrix, a measure of the parametric uncertainty is obtained and can be used to reflect the confidence in the process model predictions [112]. The $\mathbf{R}_v$ matrix can be derived from the error statistics of the measurement devices [122] and the $\mathbf{P}$ matrix is updated as in [111].

In real-time simulation, two consecutive optimization problems are solved. First, by solving the MHE problem, missing state variables are inferred and measured state variables are estimated from noisy data. Second, MPC determines the optimal feeding strategy based on these estimated variables. A schematic representation of the real-time implementation of NMPC and MHE is shown in Fig. 3.2.

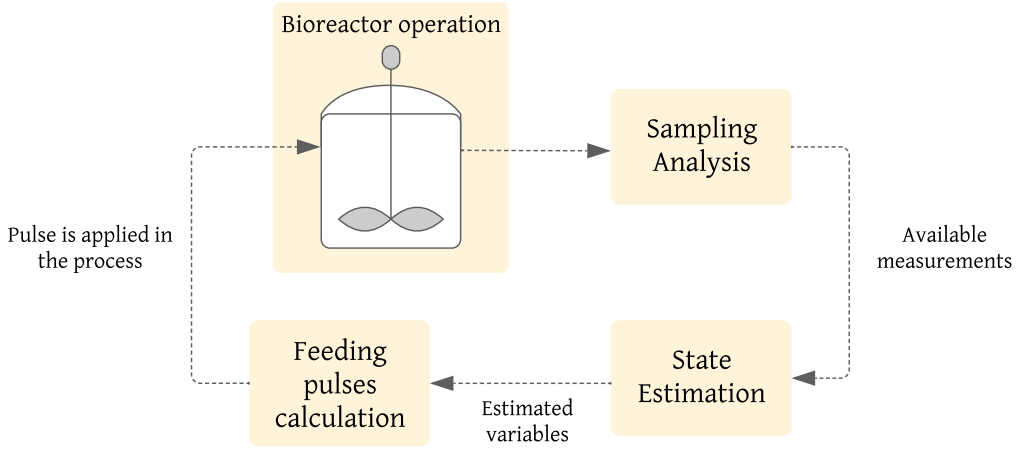


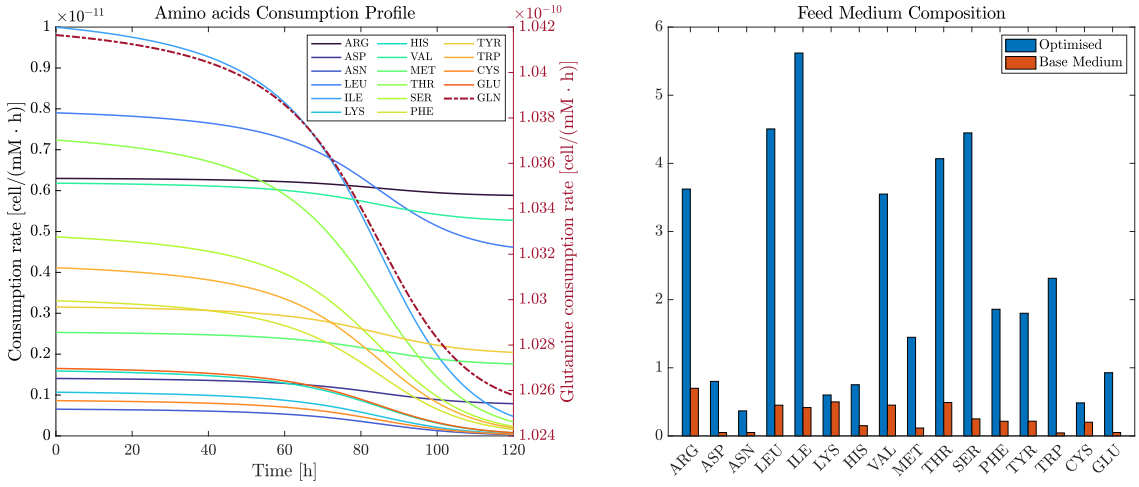
Figure 3.2: Schematic representation of the MPC-MHE framework. During the culture, measurements are taken at regular intervals, analyzed, and used to infer missing state variables and estimate measured variables from available data. These estimates are then used in subsequent MPC calculations to determine the optimal feeding strategy.

3.4. Results and discussion

3.4.1. Optimised Media Composition

The proposed model is a crucial step in developing and optimizing the medium design and feeding strategy since it predicts the nutritional requirements of the culture and its future states. These models are designed to monitor and control critical process parameters (CPPs) [25, 84]. They facilitate understanding the impact of process parameters on critical quality attributes (CQAs) and in identifying and managing sources of variability. As a result, the manufacturing process can be continuously monitored and optimized to maintain consistent product quality. This approach aligns with the principles of Quality by Design (QbD), which emphasizes a systematic strategy for ensuring product quality.

Glutamine was selected because of its role as one key energy source in the tricarboxylic acid (TCA) cycle [19, 123] and its elevated consumption rate throughout the batch culture. The specific consumption rates observed at 24 hours were selected to calculate this ratio due to the abundant availability of nutrients in the culture. During this period, cells exhibited a relatively constant consumption rate as shown in Fig. 3.3.a, thereby minimizing the potential effects of nutrient starvation. The medium composition obtained is presented in Fig. 3.3.b.



(a) (b)

Figure 3.3: Medium Design Strategy: (a) Simulation results for amino acid consumption profiles in batch mode. The dashed line represents the specific consumption rate of glutamine and the solid lines represent the specific consumption rate of the rest of the amino acids. (b) Comparison between optimized medium composition and base medium. The blue bars represent the optimized medium composition, while the orange bars represent the base medium composition.

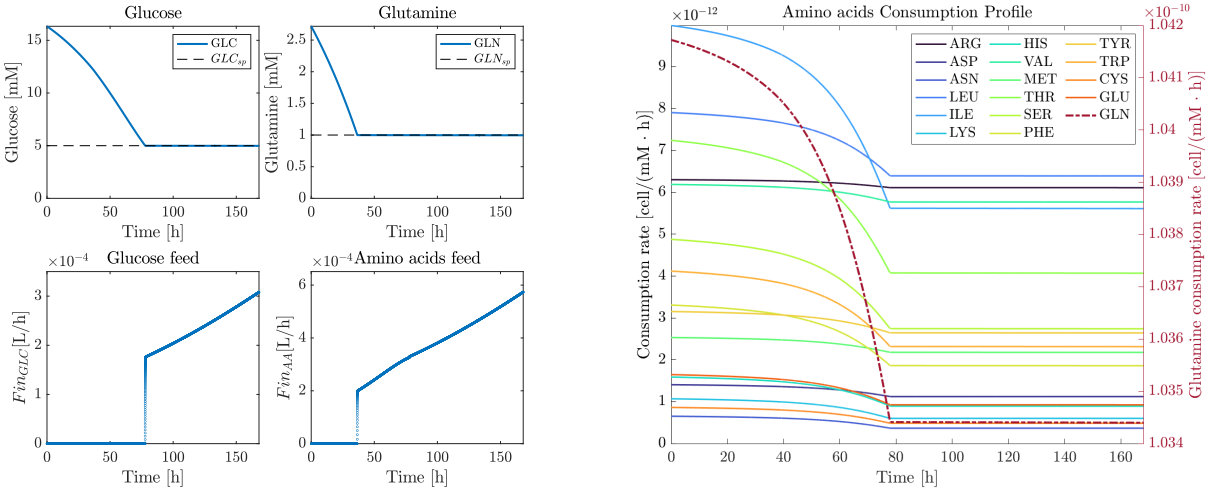
This step is crucial for addressing the control problem. Reducing the number of decision variables reduces the complexity of the optimization problem, from 21 critical process parameters (20 amino acid feed rates and glucose) to exclusively controlling glucose and glutamine. Furthermore, this methodology eases the implementation of the control scheme on a laboratory or industrial scale, reducing the amount of equipment and resources required.

3.4.2. Fed-batch Design

Proportional Control Feeding Strategy

A feedback proportional control scheme was used to estimate the feeding rates of glucose and glutamine required to maintain the controlled variables at their respective set points. To determine the feeding rate, two control loops, one for each manipulated variable, were implemented. The controller's parameters are tuned simultaneously to optimize the objective function presented in Eq. 3.5, and the fed-batch culture was simulated for 7 days.

As shown in Fig. 3.4.a despite using a simple control scheme, the system exhibits a fast response without oscillations or a significant offset. The ITAE, IAE, and ISE performance index can be seen in Table 3.4.2 and will be compared to the NMPC strategy in the next section. As expected, conventional control would not initiate control action until the process variable is below the set point. The use of a closed-loop control strategy combined with an optimized medium composition ensures the wide availability of key nutrients throughout the entire culture. By controlling only two variables, a constant specific consumption rate for all amino acids can be maintained once the set points for the controlled variables are achieved, as seen in Fig. 3.4.b. This approach prevents their depletion, avoids starvation effects, and ensures ample availability of all nutrients, not just glucose and glutamine, throughout the entire culture.



(a) (b)

Figure 3.4: P control Feeding Design Strategy: (a) Simulated glucose and glutamine profiles in the bioreactor and feeding strategy obtained in closed-loop operation. The dashed line represents the set points for glucose and glutamine. Continuous lines represent glucose and glutamine concentrations and their respective feeding rates. (b) Simulation results for amino acid consumption profiles in closed-loop operation. The dashed line represents the specific consumption rate of glutamine and the solid lines represent the specific consumption rate of the rest of the amino acids.

As shown in Fig. 3.5, the fed-batch culture led to increased biomass and a reduced number of dead cells after 120 hours of simulation. The proposed feeding strategy led to a 5.8% increase in maximum cell density at 120 [h] of cultivation. Additionally, culture longevity

improved, with cell viability increasing from 65% in the batch process to 96% in the fed-batch process at the same time, indicating a 47.7% increase. This improvement is reflected in the integral of viable cell density (IVCD), which increased from 823×10^8 cells $\cdot$ h/L to 840×10^8 cells $\cdot$ h/L over the same period compared to the batch experiment. The observed increase in IVCD highlights the effectiveness of the closed-loop feeding strategy in enhancing cell growth and reducing cell death. This reduction in dead cell concentration is primarily due to the continuous feeding of glucose, which helps avoid glucose exhaustion after 80 hours in the batch culture. Further investigation is needed in a fed-batch experiment to fully understand the effects of lactate and ammonia accumulation on cell viability beyond 120 hours of cultivation.

In fed-batch processes, metabolites such as lactate and ammonia eventually accumulate to levels that inhibit cell growth. Other factors, including high osmolarity and the accumulation of reactive oxygen species, also likely contribute to growth inhibition and lead to a decline in cell viability and productivity [124]. The effects of lactate and ammonia in CHO cultures have been widely studied, with documented impacts on growth, productivity, metabolism, and product quality [19, 123, 125]. Studies have shown that the accumulation of lactate and ammonia can induce necrotic cell death [126] and decrease the productivity of recombinant therapeutic products, as well as cause apoptosis by altering pH and osmolality [127, 128]. By minimizing the accumulation of these metabolites, the duration of the culture can be extended, achieving higher cell densities and product concentrations. Traditionally, reducing metabolite accumulation in fed-batch cultures is accomplished by controlling the availability of glucose and glutamine through feeding strategies that maintain glucose at very low levels since extended exposure to low glucose concentrations shifts cell metabolism to a more efficient state, significantly reducing lactate production [124].

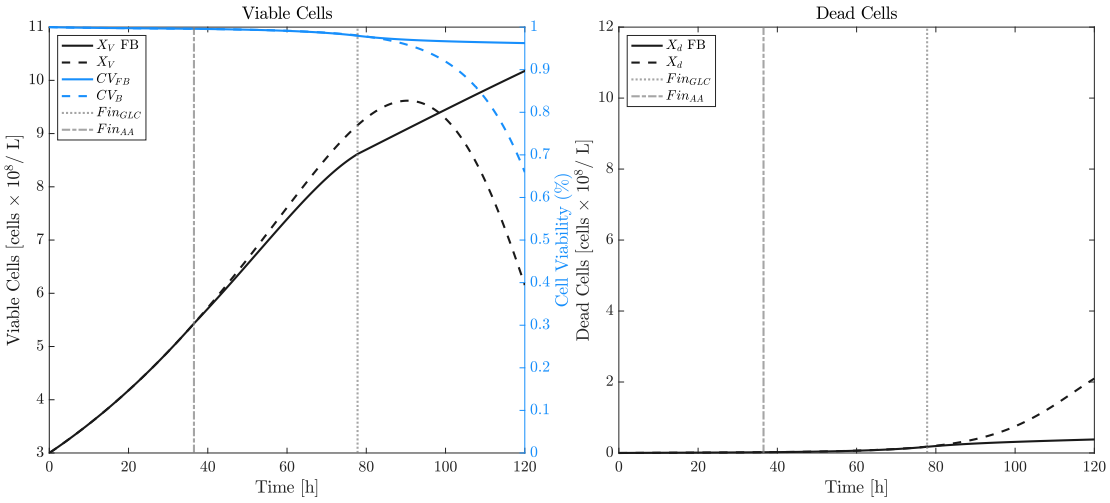


Figure 3.5: Simulated cell growth, viability and cell death in the closed-loop operation. Dashed lines represent the batch experiment, while continuous lines represent the simulated fed-batch design. Gray dashed lines indicate the time instants at which glucose and amino acid feeding begins.

However, the designed strategy does not align with the reactor operation. Therefore, it is necessary to adapt the continuous feeding strategy to the specific setup of the experi-

ment. This adaptation involves designing a pulse feeding strategy that meets operational constraints, including specific feed-time schedules and the duration of the applied pulse. These constraints could also be applied to an industrial setting, representing time shifts based on worker's availability, maintenance schedules, and specific production requirements. The pulse approximation and open-loop simulation are shown in Fig. 3.6.

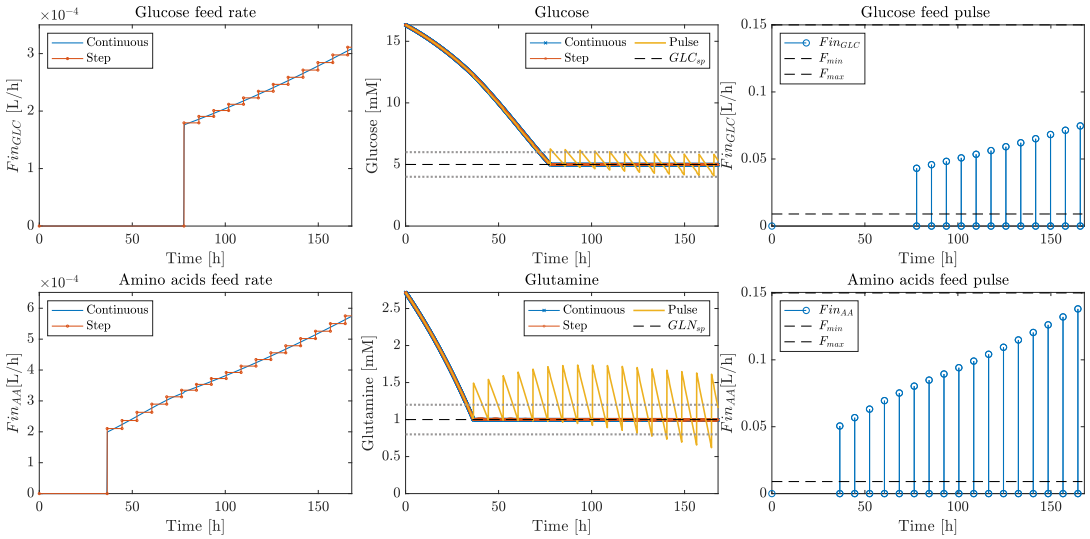


Figure 3.6: P control Pulse Feeding Strategy: Continuous feed rate approximation from the P control feeding strategy. The blue lines represent the feeding rates of glucose and glutamine, while the red lines indicate the step approximation (left). Simulated glucose and glutamine profiles in the bioreactor using the approximated pulse-feeding strategy. The curves for glucose and glutamine are colored as follows: yellow for pulse feeding, blue for continuous feeding, and red for the step feeding. The dashed lines represent glucose and glutamine setpoints (center). Glucose and amino acid feed pulses were determined from the approximation of the P control feeding strategy. The dashed lines represent the minimum and maximum flow rates allowed by the pump (right).

Despite good performance in closed-loop, the approximated feeding profiles and open-loop control strategy utilized fail in several aspects: (i) The control strategy is implemented such that the controlled variables are maintained above their set points rather than within their neighborhood. (ii) The performance decreases over time due to errors from the approximation used, namely truncation errors, causing deviations in the glutamine concentration from its set point. (iii) By design, the controller cannot handle hard constraints like maximum allowed flux, which would require manually adjusting the pulses. These types of inconsistencies in the control strategy can lead to both overfeeding and underfeeding. In particular, overfeeding can lead to metabolic imbalances and affect product yields, as observed in studies where cultures were overfed with glucose, resulting in altered final cell densities and titers [129] and inefficient nutrient utilization leading to the accumulation of toxic byproducts [130]. Overfeeding or undersupplying nutrients can also have more profound effects in the later stages of culture, as the errors accumulate over time. For example, the depletion of a particular

amino acid can cause amino acid misincorporation in the protein product, where the correct amino acid in short supply is replaced by an incorrect one, potentially affecting the quality and functionality of the final product [39]. These limitations highlight the need for further refinement and adaptation of the control approach to effectively manage these constraints and improve overall system performance. Therefore, a model predictive control strategy was employed to overcome these limitations.

NMPC Feeding Strategy

For the NMPC fed-batch design, the strategy is evaluated in both pulse and continuous feeding scenarios and compared to the proportional control strategy. The NMPC tuning parameters were experimentally adjusted, considering an 8-hour prediction horizon and a 1-hour sampling time chosen due to the relatively slow dynamics of the process. Increasing the sampling time to 12 hours did not adversely affect the NMPC controller's effectiveness, and similar results were observed with a shorter horizon (data not shown). As shown in Fig. 3.7, with proper tuning, NMPC can achieve results comparable to those of closed-loop P control when using a continuous feed. The total absolute error for continuous feedings using both P and NMPC control strategies was 0.04% for glucose and glutamine feedings, demonstrating the similarity between the strategies. Additionally, the performance criteria for both controllers were equivalent, as presented in Table 3.4.2. This equivalence indicates that, under the simulated conditions, both control strategies are equally effective in meeting control objectives when applying a continuous feed. This similarity also translates to equivalent biomass growth in both cases.

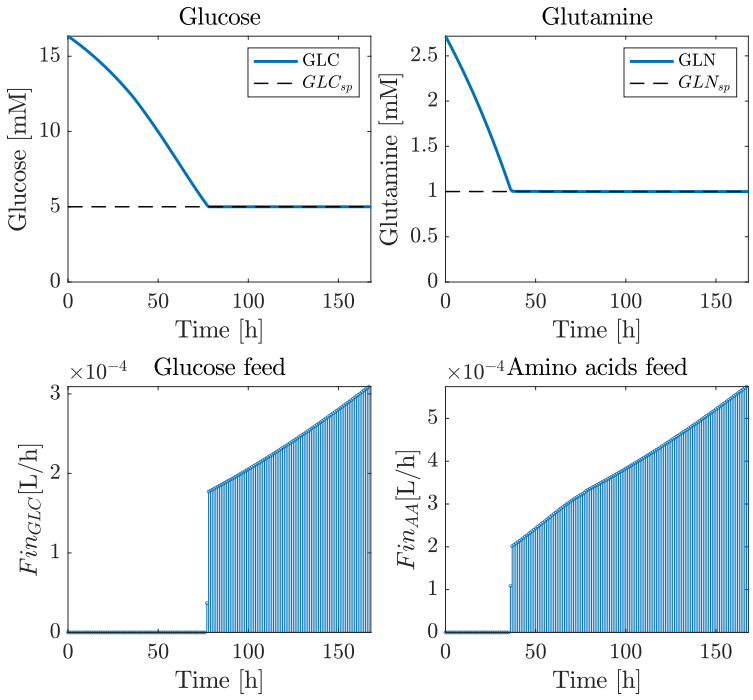


Figure 3.7: Simulated feeding strategy using NMPC for the continuous feeding case is shown at the top. Continuous lines represent glucose and glutamine concentrations, while dashed lines indicate the set points for glucose and glutamine. The bottom panel shows the optimal glucose and glutamine feed rates.

In the pulse-feeding scenario compared to the P control strategy, utilizing NMPC with constraints allows for the determination of the optimal feeding strategy while maintaining glucose and glutamine concentrations within a set point range. This approach takes into account equipment limitations and operational constraints determined by the specific feeding times during optimization. Furthermore, NMPC offers intuitive parameterization by adjusting a process model, including tuning the $\mathbf{Q}$ and $\mathbf{R}$ weighting matrices, though it comes at a higher computational effort cost than classical controllers [75].

The same previous horizon of 8 hours was considered with a sampling time of 1 hour. The weights of the $\mathbf{Q}$ matrix of the NMPC objective functions were manually adjusted by trial and error to avoid feeding at earlier stages of the culture. The simulated feeding strategy for the NMPC strategy with pulse feeding is shown in Fig. 3.8. Compared to the P control strategy presented in Fig. 3.6, the controlled variables in the NMPC approach remain nearer to their respective set points. Specifically, glutamine concentrations do not exceed 1.5 mM, while glucose concentrations are maintained within 20% of their reference value. Unlike the P control strategy, where pulses are injected separately, both pulses are injected together at 8-hour intervals. This helps to mitigate the impact of volume changes on the concentration of both nutrients when fed separately. NMPC for the pulse strategy achieves lower ITAE, IAE, and ISE criteria values compared to the P control strategy (see Table 3.4.2). The lower values for these criteria with NMPC suggest it handles errors more effectively across different aspects like time, magnitude, and duration compared to the P control strategy [131].

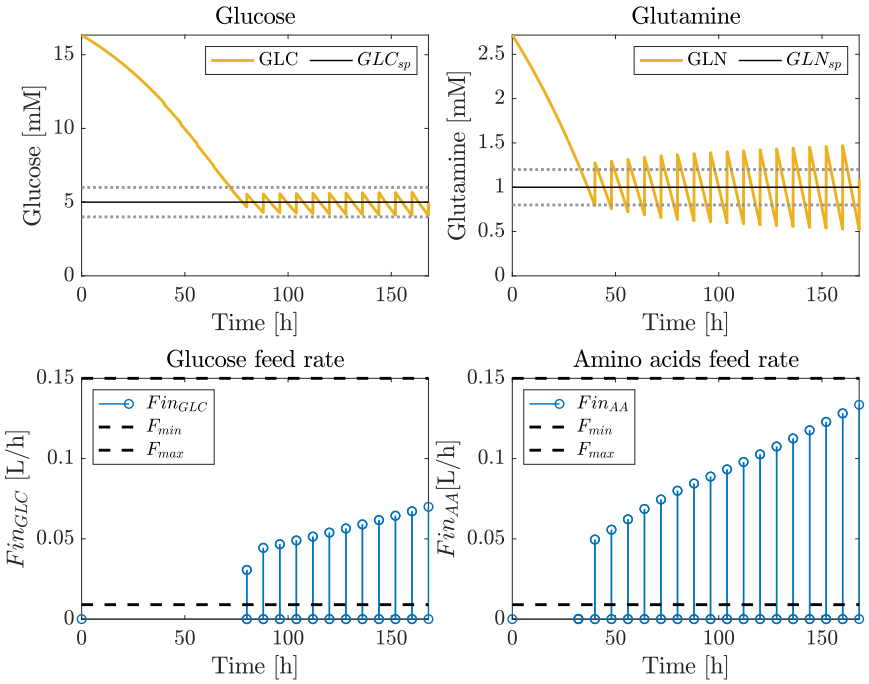


Figure 3.8: Simulated feeding strategy for pulse-feeding NMPC with constraints: Continuous lines represent glucose and glutamine concentrations, while dashed lines indicate the set points for glucose and glutamine. The bottom panel shows the optimal glucose and glutamine feed pulses.

Since the mathematical model was developed from a batch experiment for the fed-batch design, it is crucial to account for differences in cell culture between batch and fed-batch operations [25]. Accumulation of toxic metabolites, growth inhibition, and starvation effects due to increased biomass, which are not observed during batch operation, could lead to inaccurate predictions of fed-batch performance. Consequently, relevant parameters of the model may need to be re-estimated for further optimization to mitigate these effects. Moreover, open-loop control is generally effective when the process follows stable trajectories with a sufficiently accurate predictive model and there are no significant distortions [42]. In cases where significant deviations occur—such as model inaccuracies, including process-model mismatch, incorrect models, poorly fitted parameters, or external disturbances—early corrections are essential to prevent critical issues like key nutrient exhaustion. These scenarios will be addressed in the next section on real-time simulation using NMPC.

Table 3.2: Comparison of the total error-integral performance indexes in the continuous and pulse feeding strategies between the P and NMPC controllers.

	Continuous		Pulse	
Index	P	NMPC	P	NMPC
ITAE	1.40×10^4	1.39×10^4	2.29×10^4	2.10×10^4
IAE	5.31×10^2	5.31×10^4	6.12×10^4	5.91×10^2
ISE	4.11×10^3	4.11×10^3	4.14×10^3	4.13×10^3

3.4.3. Real-time simulation using NMPC-MHE.

The final section of this work assesses the feasibility of implementing the proposed NMPC framework in real-time. This study aims to replicate a scenario where precise measurements may be scarce and not all state variables can be measured. In practice, most states cannot be measured online due to the high cost of equipment and maintenance or the absence of online devices. Relevant variables like amino acids or ATP, which significantly impact process performance, are among these states [132].

Among the analytical measurements available, viable cell counts, glucose, lactate, and ammonium data were considered, with the latter being measurable using a biochemical analyzer. These measurements can be taken regularly, with processing times of only a few minutes, which aligns well with the relatively slow dynamics of the system and the 8-hour fixed feeding schedule. However, glutamine is analyzed offline using HPLC, which is generally more complex and time-demanding to obtain compared to biochemical analyzers, so this variable must be estimated. Experimental data was generated from the model simulation, and sensor measurement noise was introduced by adding normally distributed error with zero mean and standard deviation of 10% the measured value.

The estimation problem's average time is 0.5 seconds, while the control problem's average is 11.5 seconds. The total duration of a 7-day simulation was 403 seconds. Applying pulses at fixed time intervals helps minimize the processing time. Notably, the average time is potentially shorter than the sampling interval, suggesting that the optimization could be efficiently solved and executed in real time applications.

The same NMPC tuning parameters were considered, and an estimation horizon of 8 hours was used for the estimation problem. A large prediction horizon is required since pulses are applied at a pre-defined schedule every 8 hours. The obtained feeding strategy and state estimates in the presence of measurement noise are presented in Fig. 3.9. From the simulation, it can be observed that the estimates for glucose, lactate, and ammonia are accurate despite the presence of noise in the experimental measurements. Additionally, using the measured variables, it is possible to determine the concentration of glutamine even without having experimental data for this variable. Similar to the previous NMPC feeding strategy, glucose and glutamine maintain a trajectory around their respective set points, and the feeding pulses remain within the acceptable ranges for the pump.

The proposed MHE approach allows for accurate estimation of glutamine concentration in the culture despite the absence of direct measurements and provides a correct estimation of the rest of the state variables subject to measurement noise. The NMPC-MHE framework effectively addresses the limitations of previous open-loop control strategies by incorporating information from available state variable measurements, allowing for real-time adjustments and monitoring of variables that are not directly measurable. This approach ensures effective control without requiring direct glutamine measurements, which are challenging to obtain. Additionally, the feedback control mechanism within the framework helps mitigate system disturbances, leading to robust and effective process management.

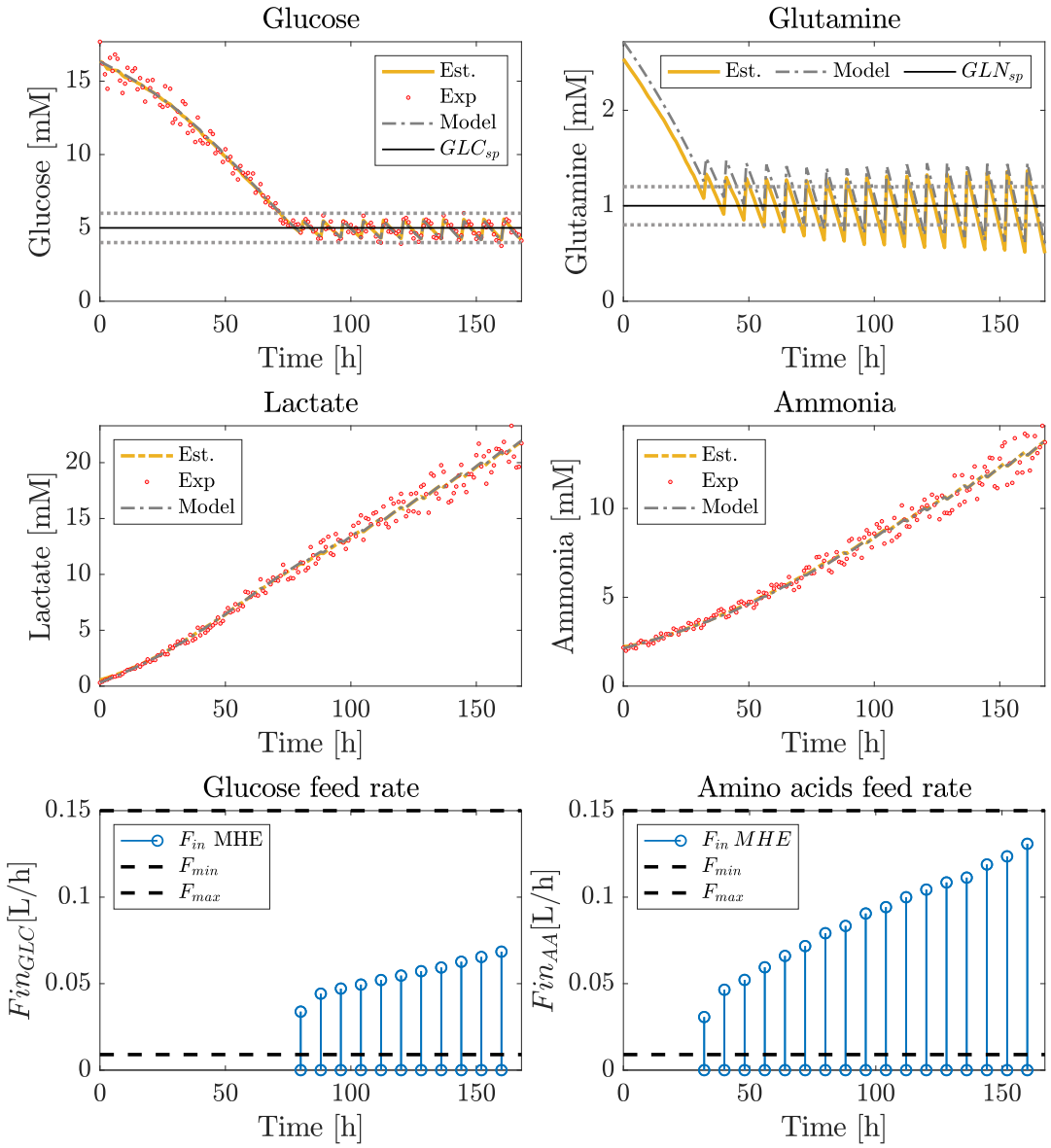


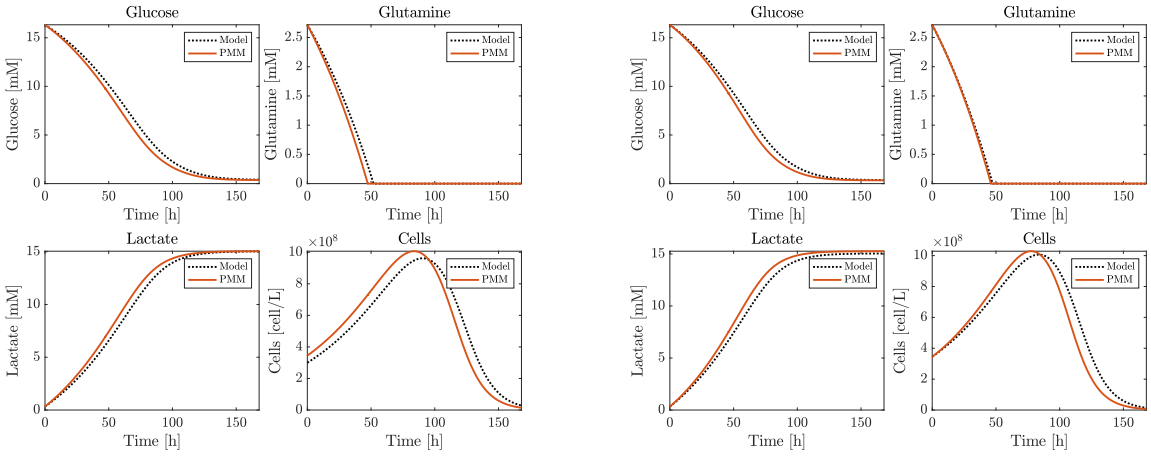
Figure 3.9: Real-time simulation of the NMPC-MHE control strategy. Glucose and Glutamine: The points represent experimental measurements. The gray dashed line indicates the model simulation. The yellow curves depict the estimated concentrations of glucose and glutamine, while the solid black line represents the setpoints for glucose and glutamine (top). Lactate and Ammonia: The points represent experimental measurements. The gray dashed line indicates the model simulation. The yellow curves depict the estimated concentrations of lactate and ammonia (center). Glucose and Glutamine Feeding: Optimal glucose and glutamine feed pulses (bottom).

Effect of perturbations on the model and feeding strategy

Creating a perfect bioprocess model is nearly impossible due to inherent variability. Consequently, real-time NMPC applications will inevitably encounter process-model mismatches [100]. With the developed model, disturbances such as variability in inoculum size, initial nutrient concentrations, feeding media composition, and changes in volume can be simulated, allowing their impact to be studied and addressed. However, disturbances in pH, temperature, and dissolved oxygen are controlled independently, so these variables are not directly considered in the mathematical model. To assess the NMPC-MHE controller performance, and response to variability of the process, two types of disturbances were introduced and evaluated. To highlight the differences between real-time monitoring and the NMPC open-loop feeding strategy, both scenarios were compared under these perturbations.

The first type of disturbance is process-model mismatch, where the model used for predictions and estimations deviates from the actual process dynamics. In this case, cell growth dynamics are impacted by a 10% increase, which may be due to unaccounted changes in the cells' physiological conditions. These conditions include variations in pH levels, oxygen concentration, temperature, or cell density [39]. Such discrepancies between the model and the actual process can lead to significant differences in the quality attributes of the product across different runs. Addressing these unmodeled variations is crucial for maintaining consistent product quality and ensuring that the control strategy remains effective despite these disturbances. The second type of mismatch considers that the process and the predictive model share the same dynamics, but at the moment of inoculation, the initial inoculum contained $\pm 15\%$ more cells than stipulated. Such perturbations reflect common operational scenarios in bioreactor operation and can significantly impact the availability of glucose and glutamine in the culture.

The effect of the aforementioned disturbances in the batch experiment can be observed in Fig. 3.10.a and Fig. 3.10.b. Overall, a larger number of cells and an increased specific growth rate would lead to earlier nutrient depletion and lactate accumulation at the beginning of the culture. In the case of glutamine consumption, although parameter fitting from the fed-batch experiment suggested that its concentration does not significantly affect the specific growth rate μ, glutamine remains crucial for other essential cellular functions. It plays a key role in energy production through the TCA cycle and serves as an important source of carbon and nitrogen [13]. Therefore, maintaining its availability throughout the entire culture is critical for optimal bioprocess performance.



(a) (b)

Figure 3.10: Effect of perturbations on the state variables of the system. (a) An error of 15% was introduced in the initial concentrations of viable cells. (b) An error of 10% was introduced to the specific growth rate μ. Simulations were performed using the model fitted to experimental batch data.

The real-time simulation of the NMPC-MHE controller with process model mismatch is shown in Fig. 3.11 and compared to the open-loop strategy. During the first 40 hours of culture, both experiments show similar behavior due to the ample availability of glucose. The main differences can be observed from hour 72 onwards, where the NMPC-MHE strategy starts feeding 8 hours earlier than the open-loop strategy. Following the predefined feeding strategy, glucose concentration drops below the desired 20% range around the set point. Similarly, despite a small offset between the measured and estimated glutamine values, the NMPC-MHE strategy ensures adequate availability of this amino acid, whereas the predefined strategy results in a considerable deviation from its set point. The most significant effect of this disturbance in the system is observed in the final concentration of viable cells, achieving 12.3×10^8 [cell/L] instead of 11×10^8 [cell/L], showing an 11.8% increase in the final biomass concentration when monitoring and controlling in real-time. The specific growth rate and viable cell concentration over time are shown in Fig. 3.12. The IVCD for the NMPC strategy was 1.37×10^3, while for NMPC-MHE it was 1.44×10^3, indicating higher viable cell density with the MHE approach. Naturally, the increased biomass growth and higher specific growth rate would require an increase in the glucose and glutamine feeds compared to the open-loop when the change in the system's dynamics is not addressed.

The integration of real-time measurements into the control strategy significantly enhances its robustness, despite mismatches between model predictions and the actual dynamics of the process. By continuously updating and correcting the control actions based on real-time data, the NMPC-MHE framework can adapt to changing conditions, ensuring optimal nutrient supply and maintaining the desired cell growth trajectory. This adaptability not only improves the accuracy of the control strategy but also has the potential to ensure the consistent quality of the bioprocess outcomes even in the presence of disturbances.

The process-model mismatch is a major issue in state estimation and model-based control

strategy. Even with well-tuned MHE parameters, the problem cannot be fully resolved [122] and when the mismatch is severe, estimation results get closer too late to be corrected [97]. To mitigate this issue, variations in model parameters need to be identified beforehand and can be incorporated as additional decision variables in the estimation problem. However, online parameter estimation can be problematic when there is a high level of signal noise or unmeasured disturbances [63]. Another important point to highlight is the variability that can arise from poor estimation of the system parameters in the initial batch experiment. Since the performance of the control system heavily depends on the predictive model, the success of the designed feeding strategy requires precise system identification and the recognition of potential sources of variability in the process.

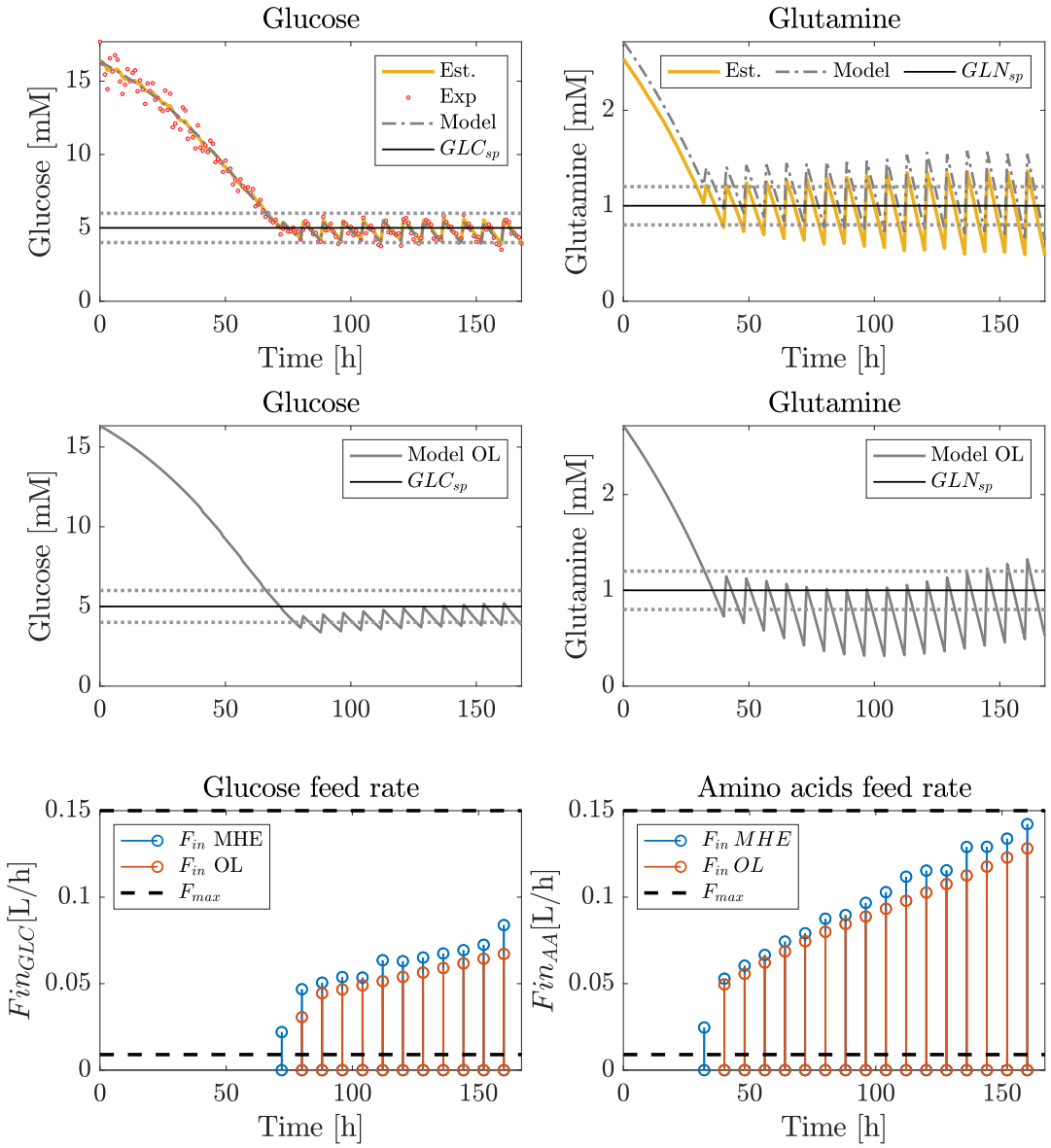


Figure 3.11: Closed-loop (NMPC-MHE) and open-loop (NMPC) strategies comparison with process model mismatch added to the viable cell dynamics. Glucose and glutamine estimated states and simulated process response in the MPC-MHE strategy (top). Glucose and glutamine simulated process response when the open-loop feeding strategy is applied (center). Glucose and amino acids pulse feed rates comparison between the closed-loop and open-loop strategies (bottom).

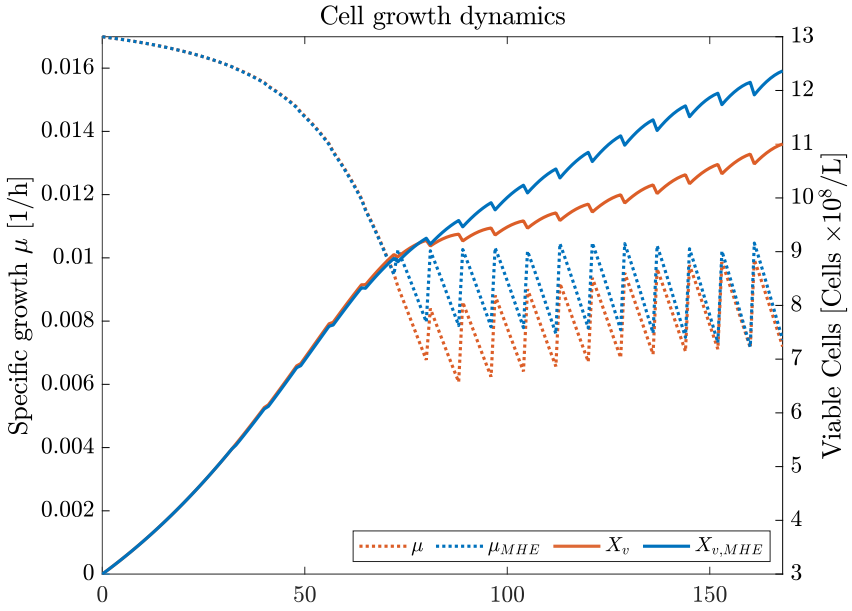


Figure 3.12: Effect on cell dynamics due to the introduced process model mismatch is illustrated in both closed-loop (NMPC-MHE) and open-loop (NMPC OL) simulations. The specific growth rate is represented by the dashed line, and the viable cell concentration is shown by the continuous line. The NMPC-MHE simulation results are depicted in blue, while the NMPC OL simulation results are shown in orange.

The second disturbance represents an error during inoculation. For an industrial fed-batch process this could be seen as batch-to-batch variations in feed concentration or initial biomass concentration [35]. In practice, this mistake would necessitate adjusting the feeding strategy based on the magnitude of the error, but this could be done only if it is measured in advance. This situation increases the risk of inaccuracies and may result in suboptimal control actions, potentially affecting the overall performance of the process. In the context of the MHE problem, this situation represents a bad prior estimation of the initial states which could lead to suboptimal control actions. The real-time *in-silico* experiment with a 15% increase in initial concentration, shown in Fig. 3.13, illustrates that, despite initial glutamine estimation errors, MHE can adjust predicted values as more measurements are incorporated.

Unlike the open-loop strategy, where glucose concentration fails to reach the desired set point until hour 120 and glutamine drops to critical levels early in the culture—potentially causing irreversible effects on cell growth and metabolism—the adaptive feeding approach of MHE ensures earlier feeding of glucose and glutamine pulses in response to increased biomass growth. Ghaffari *et al.* [133] found that the absence of key nutrients like asparagine and glutamine caused a significant reduction in cell growth. Specifically, glutamine depletion alone resulted in about a 40% decrease in final product concentration and over a 30% reduction in cell-specific productivity for CHO-S cells. The simulations and findings underscore the critical importance of amino acid availability and real-time monitoring in optimizing fed-batch cultures. They also highlight the significance of the media design step, which is fundamental for aligning nutrient supply with the metabolic demands of the cells.

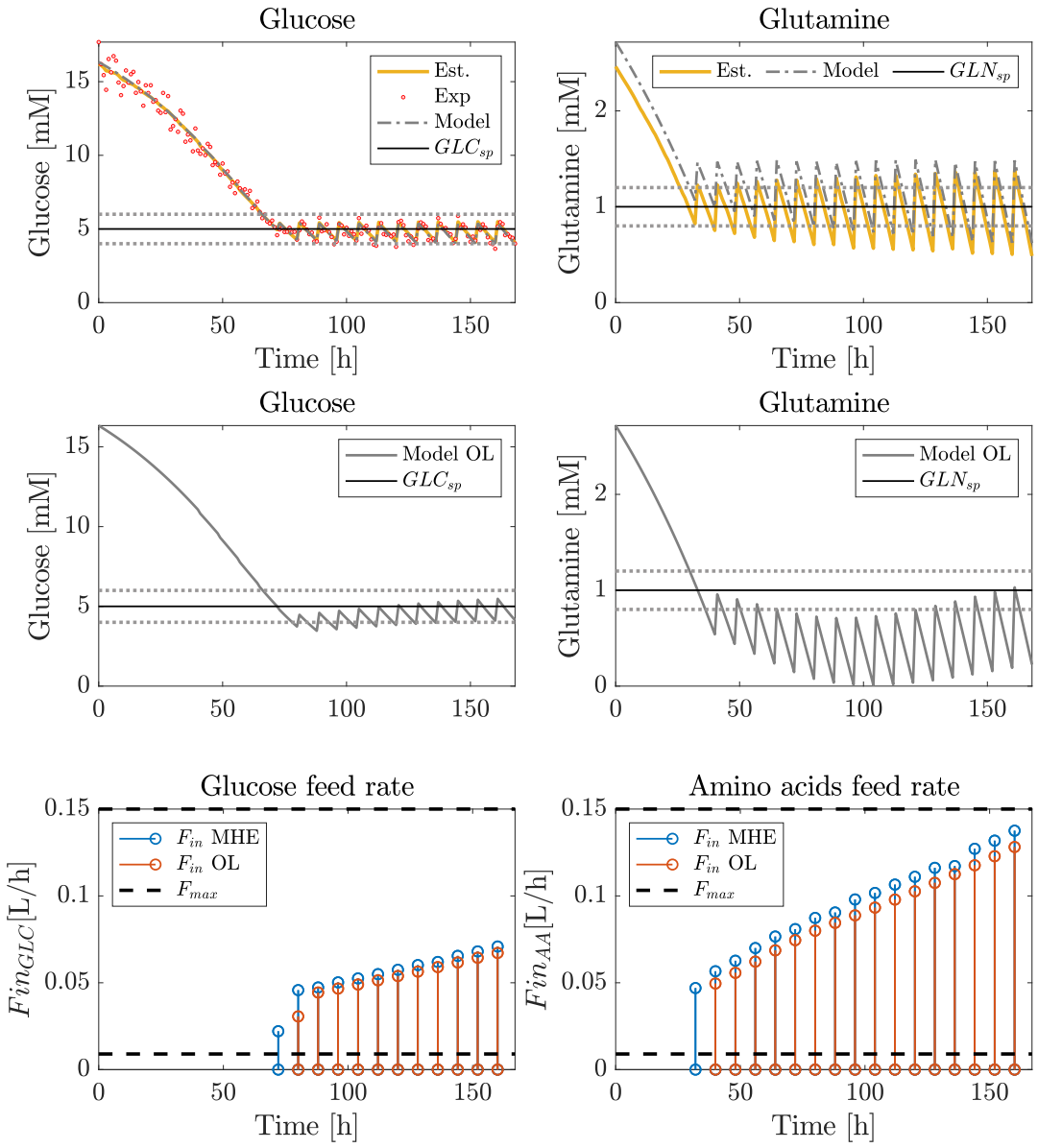


Figure 3.13: Closed-loop (NMPC-MHE) and open-loop (NMPC) strategies comparison. An error of +15% was introduced in the initial concentrations of viable cells. Glucose and glutamine estimated states and simulated process response (top) in the MPC-MHE strategy. Glucose and glutamine simulated process response when the open-loop feeding strategy is applied (center). Glucose and amino acids pulse feed rates comparison between the closed-loop and open-loop strategies (bottom).

A similar analysis can be conducted when the initial inoculum is lower than the amount used during the design of the feeding strategy, as shown in Fig 3.14. In this scenario, the advanced control system helps to prevent overfeeding of nutrients, thereby improving the performance of the implemented feeding strategy in terms of accurately following the reference values. Moreover, this approach facilitates a more efficient use of resources by aligning the

nutrient supply more closely with the actual needs of the culture.

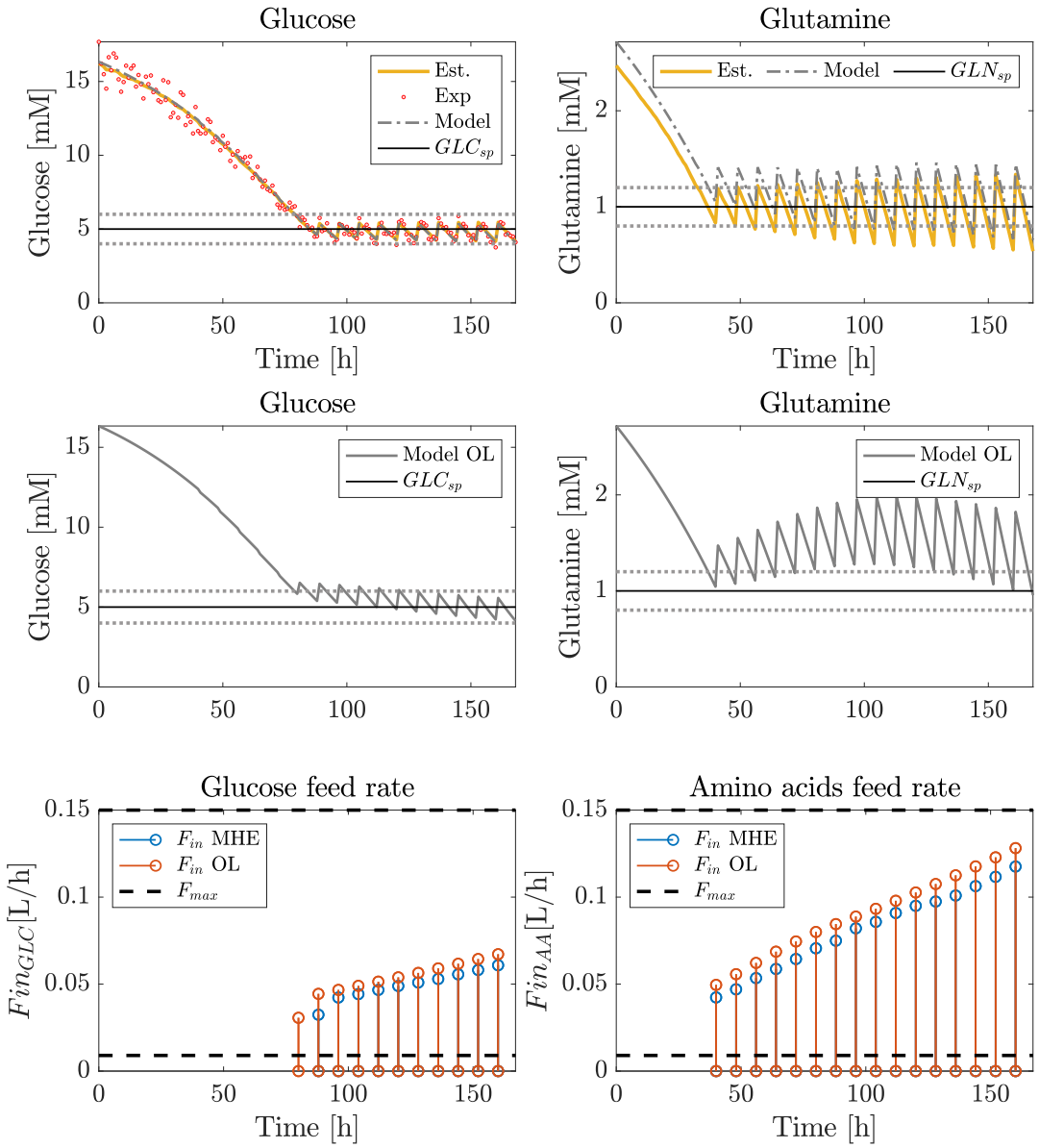


Figure 3.14: Closed-loop (NMPC-MHE) and open-loop (NMPC) strategies comparison. An error of -15% was introduced in the initial concentrations of viable cells. Glucose and glutamine estimated states and simulated process response (top) in the MPC-MHE strategy. Glucose and glutamine simulated process response when the open-loop feeding strategy is applied (center). Glucose and amino acids pulse feed rates comparison between the closed-loop and open-loop strategies (bottom).

To justify the use of an advanced control system in industrial applications, it must provide performance improvements that outweigh its implementation costs. These improvements typically manifest as increased profitability, which can be achieved through several means:

enhanced product yields, optimized utilization of materials, and reduced operational expenses [35]. For instance, by minimizing the amount of excess nutrients and streamlining resource use, the system can lead to cost savings and better overall efficiency, making advanced control technology economically viable.

Finally, the effect of the sampling time on the system is considered. Obtaining experimental data, especially in the case of bioprocesses, can be slow and costly. To address this, the performance of the proposed NMPC-MHE control scheme was evaluated in scenarios with fewer measurements with measurement noise and process model mismatch equal to the case presented in 3.11. These measurements were considered at intervals of 2, 4, and 8 hours, and the performance was assessed based on ITAE performance criteria and the integral of viable cell density (IVCD). These results are shown in Figure B.1.

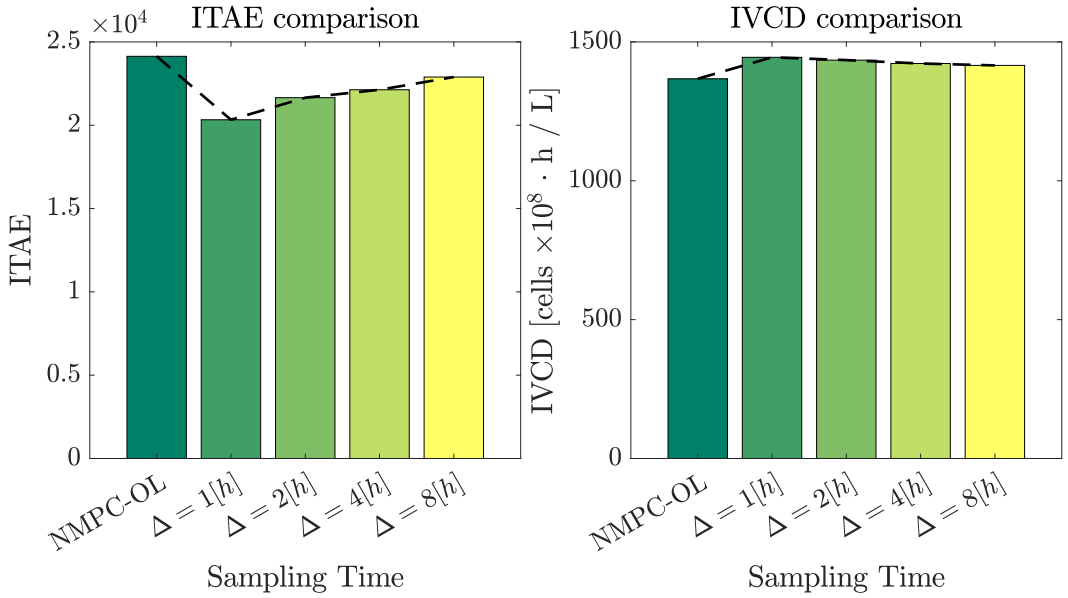


Figure 3.15: Comparison of ITAE Index and IVCD for different sampling intervals. On the left, the ITAE comparison is shown for 4 sampling intervals from 1 [h] to 8 [h]. On the right panel, the IVCD comparison is shown.

As shown in Fig. 3.15, using a frequent sampling time in real-time monitoring and control leads to increased biomass and improved control performance. With a 1-hour sampling time, the IVCD achieved is the highest, and the ITAE index is the lowest compared to all other simulated scenarios. A sampling time of up to 2 hours still yields a similar IVCD, despite the reduction in control performance in the 2-hour sampling case. This suggests that even with reduced data availability, the control and estimation problems can still be effectively addressed. Increasing the sampling time beyond 4 hours leads to a rise in the ITAE index compared to the ideal case with frequent measurements. This deviation in glucose and glutamine from their respective setpoints also results in a slightly reduced IVCD, indicating a deterioration in the overall performance of the culture. The decline in performance is primarily associated with errors in glutamine estimation. With fewer measurements available, it becomes challenging to correct the glutamine estimates accurately when no measurements of this variable are available (simulations available in Annex B). Nevertheless, the control

system's performance remains superior to the open-loop case, demonstrating a greater IVCD overall and lower ITAE. When comparing the NMPC-MHE framework with the open-loop strategy derived from NMPC, the NMPC-MHE framework consistently results in increased biomass across all scenarios, as indicated by a higher IVCD and better control performance. This highlights the critical role of monitoring and control in maintaining optimal conditions and improving the overall performance of the culture, as evidenced by the superior results achieved with the NMPC-MHE framework compared to an open-loop strategy.

In scenarios with lower sampling frequencies, glutamine tends to be underestimated, leading to higher feeding pulses for this nutrient. From these results, we can conclude that more frequent sampling (e.g., every 1 or 2 hours) is beneficial for maintaining effective control and monitoring of the system and ensuring a higher cell density. When using a larger sampling time, the control system relies more heavily on model predictions due to the reduced information available to correct potential deviations from measurements. Therefore, the accuracy of the predictive model must be carefully considered to ensure reliable performance under these conditions. While the control system can still function with less frequent sampling, performance decreases as the interval between samples increases. Thus, more frequent sampling is recommended to maintain stringent control during the process and ensure consistent outcomes. One possible solution to this issue is to increase the frequency of measurements during the early hours of the cultivation process and focus solely on the estimation problem. By doing so, accurate estimates of the controlled variables can be obtained once feeding begins, thereby ensuring effective implementation of the control strategy. However, the choice of sampling time in real-life applications will depend on the level of accuracy required and the availability of measurements, balancing the need for precision with equipment limitations, resource availability and time constraints.

Current sensors for measuring organic compounds and other parameters in biomanufacturing include electrochemical analyzers and optical sensors [134]. Electrochemical analyzers such as the Bioprofile FLEX offer automated, rapid sampling of key compounds like glucose, glutamine, lactate, and ammonia, as well as biomass and by-products, with a sampling time of up to 4.5 minutes. These analyzers are adaptable for use in both single-use bench-scale bioreactors and large production bioreactors. Additionally, Raman spectroscopy[135] is utilized for real-time monitoring of metabolites, viable cell density, and cell viability within bioreactors, providing valuable data for advanced control strategies and has been employed in various advanced control strategies in the literature, including Model Predictive Control (MPC) applications [100]. However, Raman spectroscopy requires very sensitive and highly optimized instrumentation, and for larger-scale bioprocesses, the weak signal may necessitate very expensive machinery to accurately detect variations in analyte concentrations [134].

Advancements in sensor technology have enabled more effective monitoring and control of bioprocesses, facilitating real-time decision-making and optimization. Despite these benefits, the deployment of such technologies poses challenges due to the need for substantial investment and specialized expertise [117]. Nevertheless, the adoption of these advanced tools is becoming crucial in the bioprocess industry to comply with the standards established by the PAT and QbD frameworks, prioritizing consistent product quality through real-time monitoring, control and process understanding.

3.5. Concluding Remarks

Optimal culture medium design and precise feeding strategies are crucial for maximizing biomass and bio-product production. While model-based strategies are powerful tools for media formulation and feeding optimization, their effectiveness is further enhanced when integrated with real-time monitoring, particularly in the presence of process variability and unmeasured disturbances. The proposed methodology not only reduces experimental costs, time, and resource use through extensive in-silico testing but also accelerates process development, while also providing flexibility and customization, making it applicable to various cell lines and production scenarios. The implementation of an advanced control and estimation strategy has proven effective in enhancing the accuracy of control of critical variables in a CHO cell culture, leading to improved maintenance of process variables within desired ranges and improved biomass concentration at the end of the culture compared to open-loop strategies and P control strategies.

While P control can achieve comparable performance to NMPC in continuous feeding strategies when properly tuned, it struggles with handling constraints in pulse-feeding strategies. NMPC's capability to manage operational constraints and adapt to real-world scenarios, such as worker availability and maintenance schedules, underscores its advantage in more complex and variable production environments. The significant improvement in ITAE, IAE, and ISE indices for pulse-feeding with NMPC illustrates its effectiveness and flexibility in different control scenarios.

Employing a real-time monitoring and control strategy proved effective in managing the natural variability of the fed-batch process, a challenge that open-loop strategies cannot overcome. This approach allows for process monitoring even in the absence of measurements of key variables like glutamine. The NMPC-MHE controller exhibited a lower ITAE index, indicating better accuracy in controlling the process under various disturbance scenarios. This translates to improved quality control, enhanced biomass growth, and adequate nutrient supplementation throughout the culture. The sampling time was found to have a significant impact on glutamine estimation; frequent sampling is beneficial for maintaining effective control and ensuring higher cell density. Although the control system can function with less frequent sampling, performance tends to decrease as the interval between samples increases.

Despite these advancements, several challenges remain. The precision of predictive models and the need for a deeper understanding of metabolic pathways and medium components are crucial for further improving process management. Future research should focus on several key areas: enhancing predictive models to better reflect cell culture dynamics by integrating additional experimental data, improving real-time monitoring technologies with advanced sensor systems, and refining control algorithms to handle process variability and disturbances more effectively. Evaluating different control strategies under actual operating conditions will help identify the most effective approaches in terms of productivity and cost-efficiency. By addressing these areas, future work can further optimize bioprocesses, enhance product quality, and contribute to more efficient and adaptable manufacturing practices in biotechnology.

Chapter 4

Conclusion and Future Work

In conclusion, the integration of advanced control strategies such as NMPC combined with MHE, along with real-time monitoring, can significantly enhance process control in cell cultures. This integrated approach not only improves the accuracy of control and state estimation but also leads to better maintenance of critical process variables and higher biomass concentrations at the end of the culture. The findings of this study demonstrate that both continuous and pulse-feeding strategies can achieve effective control performance when utilizing NMPC, with the added advantage of better constraint handling and adaptability to real plant scenarios compared to traditional P control.

Key results from this study include that when designing a continuous feeding strategy, both P control and NMPC control can achieve equal performance in terms of control performance as they achieve equal ITAE, IAE, and ISE indices. Despite the simplicity of the proposed P control, proper tuning can adequately manage the non-linearity of the process and the interdependency between variables.

In contrast, when designing a pulse-feeding strategy, P control struggles to handle constraints effectively, adversely affecting control performance. The ease of constraint implementation with NMPC can accommodate real plant scenarios such as time shifts based on worker availability, maintenance schedules, and specific production requirements. In the pulse-feeding strategy, ITAE, IAE, and ISE were improved from 2.29×10^4 to 2.10×10^4, 6.12×10^2 to 5.91×10^2, and 4.14×10^3 to 4.13×10^3, respectively.

The NMPC-MHE controller exhibited a lower ITAE index, indicating better accuracy in controlling the process under various disturbance scenarios. In the presence of process variability, the NMPC-MHE control scheme resulted in a 4.68% decrease in the ITAE index and a 5.34% increase in the IVCD compared to the NMPC open-loop strategy. However, sampling time was found to have a significant impact on glutamine estimation; frequent sampling is beneficial for maintaining effective control and ensuring higher cell density. Although the control system can function with less frequent sampling, performance tends to decrease as the interval between samples increases.

4.1. Future Work

One of the main challenges in implementing the proposed strategy is the rigidity in sampling times when solving the control and estimation problem, coupled with the NMPC-MHE framework's heavy reliance on the availability and accuracy of measurements. The work conducted opens avenues for further investigation into related areas of process control and estimation. Key areas for exploration include:

1. **Incorporating Measurements with Different Sampling Times:** This approach would allow the integration of "slow" measurements with "fast" measurements obtained experimentally, thereby enhancing state estimation accuracy. An example of a strategy addressing this issue is Multi-rate MHE. See [122] for a review of different strategies for multi-rate estimation focusing on MHE and the work by [97] for the challenges of its application in real-time experiments. Also, ANNs offer the capability of functioning as a soft sensor [112]. These estimators have been increasingly employed for biochemical process applications but should be trained on real data. Investigating the application of ANN in this role could provide additional benefits in estimating unmeasured variables and improving overall process control and estimation.

2. **Incorporating Robust Control Schemes:** Given the variability inherent in fed-batch processes, developing a feeding strategy capable of adequately supplementing a culture under parameter estimation uncertainty is crucial. Although this approach may result in more conservative control actions, it could represent an improvement over the proposed open-loop control strategies. See the work presented in [136–138] for examples of robust control applied to fed-batch cultures. Also in [139] an open-source software for robust model predictive control in presented.

3. **Adding New Measurements to the Mathematical Model:** While the current model relies on nutrient and cell mass balances, it can be supplemented with additional information from the culture, such as O_2 consumption and CO_2 generation. These variables are easier to measure and provide valuable insights into the culture's state, aiding in the estimation of components that are difficult to measure directly. Furthermore, incorporating these measurements can streamline the state estimation process and simplify the implementation of the control loop, reducing reliance on complex measurement techniques.

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Annexes

Annex A. Detailed description of the mathematical model

Growth Kinetics and Cell Death

Cell growth

The model assumes that cell growth depends on the availability of two key nutrients, glucose and glutamine, and the accumulation of lactate. The specific growth rate is determined by the key nutrients in the culture. These are grouped into nutrients that limit cell growth and metabolites that inhibit it in blocks. In the case of CHO cells, these limiting nutrients were glucose and glutamine, and the primary metabolite was lactate. Both relationships were determined from the analysis of batch experimental data. Therefore, the specific growth rate can be formulated as:

$$\mu = \mu_{max} \cdot \left(\frac{1}{1 + \left(\frac{K_{glc}}{GLC}\right)^{n_{g,glc}}} + \frac{1}{1 + \left(\frac{K_{gln}}{GLN}\right)^{n_{g,gln}}} \right) \cdot \left(\frac{1}{1 + \left(\frac{LAC}{K_{i,lac}}\right)^{n_{g,lac}}} \right) \tag{A.1}$$

Where GLC, GLN, and LAC are the glucose, glutamine, and lactate concentrations in the culture medium, while K_{glc}, K_{gln} denotes the Monod's growth constants and $K_{i,lac}$ the Monod's inhibition constant. The influence of each variable in growth and inhibition is determined by its Hill coefficient n_i.

Cell death

The specific death rate μ_c is composed of the starvation effect STV and toxic effects due to lactate accumulation. The STV term describes the inhibition of cell growth in the absence of key nutrients such as glucose and glutamine.

$$STV = \left(\frac{1}{1 + \left(\frac{GLC}{K_{lim,glc}}\right)^{n_{d,glc}}} \right) \quad \mu_d = \mu_{d,max} \cdot STV + \mu_{d,lac,max} \cdot \left(\frac{1}{1 + \left(\frac{K_{i,lac}}{LAC}\right)^{n_{d,lac}}} \right) \tag{A.2}$$

Where $K_{lim,glc}$ is the specific death rate from lactate toxicity, $K_{i,lac}$ is the Monod constant for metabolic inhibition from lactate and n_d, i its Hill coefficients.

Glucose and Lactate Metabolism

The specific glucose consumption rate is divided into three terms describing the consumption of this nutrient for cell proliferation $Q_{x,glc}$ and energy production during glycolysis $Q_{glyc,glc}$ and the TCA cycle $Q_{k,glc}$. Each consumption term follows the next equations:

$$Q_{i,glc} = \frac{\mu}{Y_{i,glc}} + M_{i,glc} \tag{A.3}$$

Where $Y_{i,glc}$ represents the yield of biomass from glucose and the yield of biomass from energy from glucose on its respective consumption terms and $M_{i,glc}$ their maintenance coefficients. Assuming that pyruvate does not accumulate, a glucose molecule can produce lactate and alanine or enter the TCA cycle via acetyl-CoA formation. Lactate is produced during glycolysis $Q_{glyc,glc}$ at a stoichiometric rate, and for every molecule of glucose that completes glycolysis and forms lactate, two lactate molecules are produced. $R_{lac,ala}$ denotes the proportion between lactate and alanine produced from glucose consumption during glycolysis. Finally the specific consumption rate of glucose Q_{glc} and specific production rate of lactate Q_{lac} can be written as:

$$Q_{glc} = \sum_i Q_{i,glc} \qquad\qquad Q_{lac} = 2R_{lac,ala}Q_{gly,glc} \tag{A.4}$$

Glutamine and Ammonia Metabolism

Similarly to glucose, the specific consumption rates can be separated into terms describing nutrient consumption used for proliferation $Q_{x,gln}$, energy production $Q_{k,gln}$, and product synthesis if applicable. $Y_{amm,gln}$ represents the yield of ammonia from glutamine. Also, in the glutamine mass balance, a degradation term is considered $K_{d,gln}$, representing glutamine degradation on the medium. Similarly to Eq. A.4 and following the same structure of Eq. A.3, the specific consumption rate of glutamine and specific production rate of ammonia can be written as:

$$Q_{gln} = \sum_i Q_{i,gln} \qquad\qquad Q_{amm} = Q_{k,gln}Y_{amm,gln} \tag{A.5}$$

The specific consumption rates of the rest of the amino acids Q_{aa} follow the same structure as those of glutamine, considering amino acid consumption for proliferation $Q_{x,aa}$, energy production $Q_{k,aa}$ in the case of amino acids that enter the TCA cycle.

Annex B. Effect of sampling time in the control and estimation problem

The effect of the sampling time without process model mismatch is shown in Fig. B.1 in term of control performance and IVCD obtained at the end of the process. Using a sampling time of 1 or 2 hours allows for equivalent control performance and IVCD. This suggests that with less data availability, the control and estimation problems can still be addressed optimally. However, increasing the sampling time beyond 4 hours leads to an increase in the ITAE index compared to the ideal case with frequent measurements.

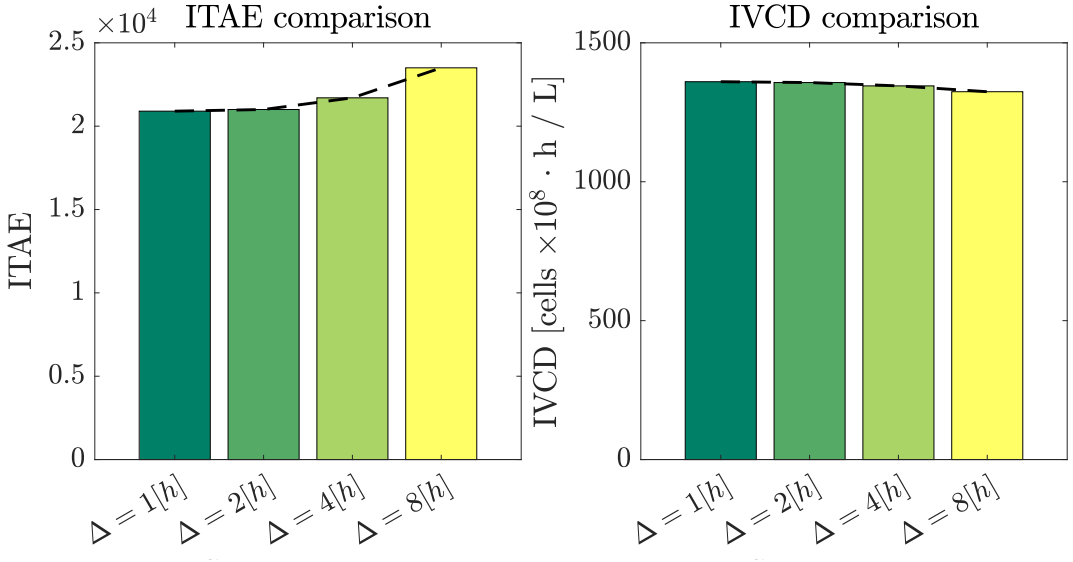

Figure B.1: Comparison of ITAE Index and IVCD for different sampling intervals. On the left, the ITAE comparison is shown for 4 sampling intervals from 1 [h] to 8 [h]. On the right pannel the IVCD comparison is shown.

The effect of sampling time in the model's predictions is shown in Fig. B.2 - B.4. A sampling time of 2 to 4 hours can effectively estimate the glucose concentration from noisy data. In the case of a sampling time of 8 hours, it can be observed that glucose estimates diverge from its desired setpoints. The estimation of glutamine presents a significant challenge, as its values cannot be corrected with experimental data unlike glucose. The initial offset in glutamine estimates cannot be adjusted based on observed measurements from available data.

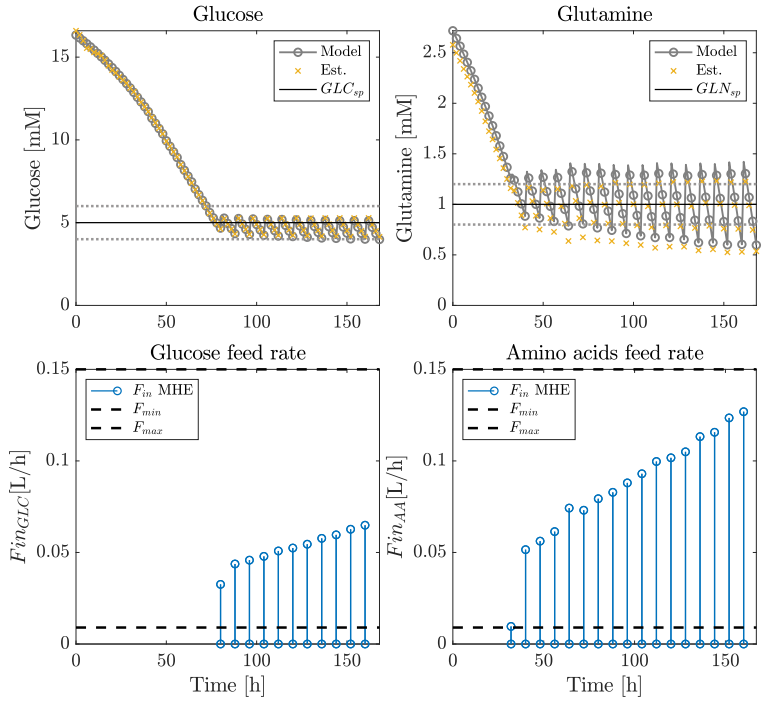

Figure B.2: NMPC-MHE simulation with sampling time of 2 hours.

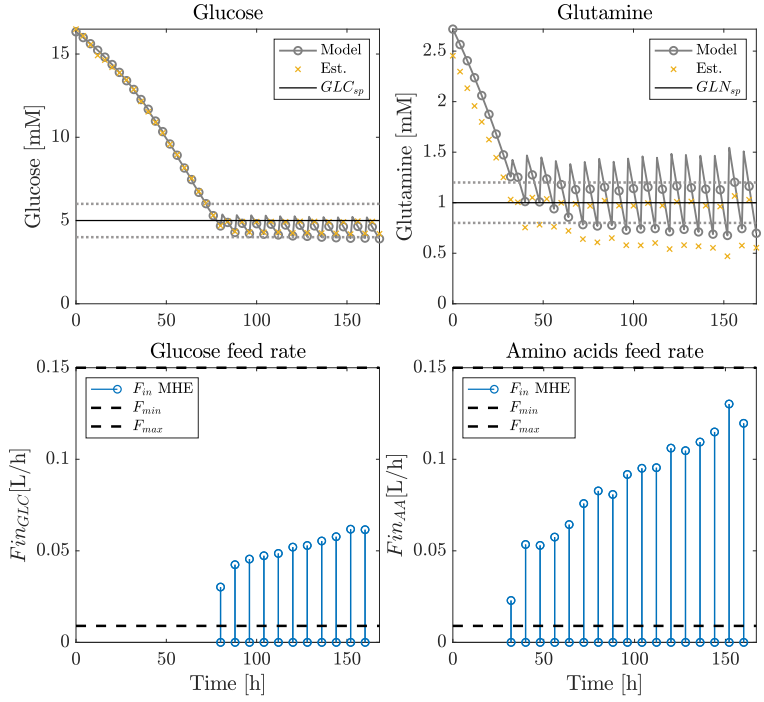

Figure B.3: NMPC-MHE simulation with sampling time of 4 hours.

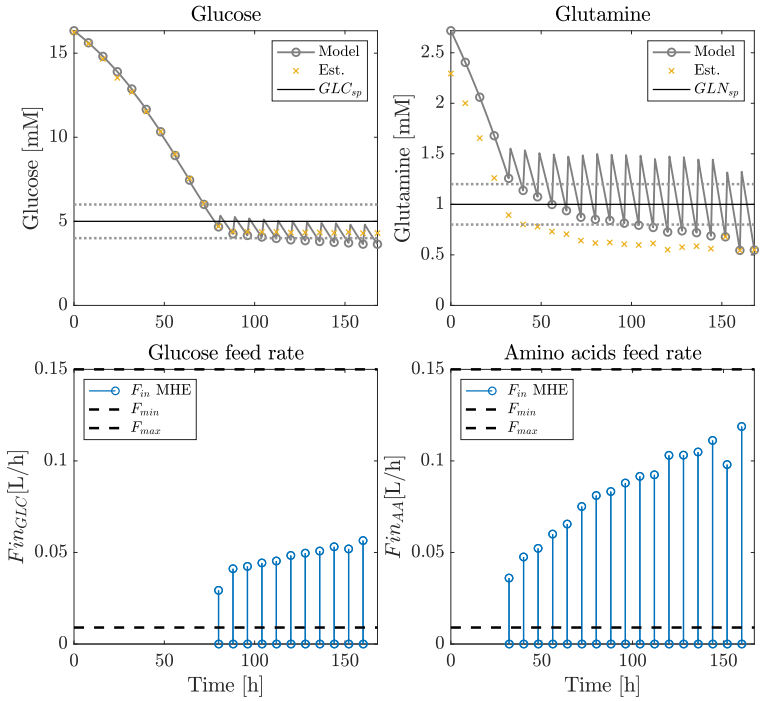

Figure B.4: NMPC-MHE simulation with sampling time of 8 hours.