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Chapter

Fermentation: Metabolism, Kinetic Models, and Bioprocessing

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Abstract

Biochemical and metabolic interpretation of microbial growth is an important topic in bioreactor design. We intend to address valuable information about the relation of critical operation variables and the simulation of bioprocesses with unstructured and structured kinetic models. Process parameters such as nutrient supply, pH, dissolved oxygen, and metabolic end-products directly impact the physiology and metabolism of microorganisms. Changes in the membrane as well as cell viability are of interest since protein expression and maturation in prokaryota are directly related to membrane integrity. This chapter intends to deliver an insight of different alternatives in kinetic modeling.

Keywords: metabolism, unstructured kinetic models, black box models, gray box models

1. Introduction

Bacteria are the dominant form of life spread across the whole planet. Their biochemistry machinery is well adapted to scarcity conditions; also, they can biosynthesize complex molecules in various environmental conditions. For this reason, the growth and proliferation of bacteria in controlled environments represent an interest of biochemical engineers, microbiologists, and cell-growth enthusiasts since they allow bioprocess simulation and control scheme design. Substrate transformations into cell biomass, organic molecules, therapeutic proteins, biofuels, enzymes, and food additives are of attention since application to actual fields and laboratory experiments are very difficult to scale-up to industrial level with strict and complete control of key variables determined as an ideal process [1]. It is known that the complexity in a mathematical model may increase with the inclusion of environmental conditions such as multisubstrate consumption and product formation, pH change during fermentation, variable temperature, rheological changes in culture media, multiphasic environmental variability, and nonideality of mixing and stirring [2]. The kinetic model had been evolved from simple exponential growth to complex mathematical expressions to predict heterogeneity in single cells, describe multiple reactions, explain internal control mechanisms, and even predict genetic variability between bacterial populations [3]. However, despite the efforts to represent the progress of biological reactions in microbial cultures, the actual application of the model in real production processes is impractical due to a significant amount of information fed to the model [4].

Many of the kinetic growth models base their structure on and take information from empirical observations through experimental data. The white box models (WBMs) use information from mass balances in a single stoichiometric equation where inputs, outputs, and the conversion from substrates to products are followed [5]. Despite effectiveness and advanced reaction representation in WBMs, the representation of the reaction advance degree, some information on metabolic flux analysis (MFA) can be obtained. Models based on detailed MFA can be used to define optimal operation conditions based on biochemical pathways. It has been established that kinetic models of biological reactions are more complicated than "common" chemical reaction models. Microbial growth models require specialized knowledge of rapid changes of environmental conditions, stoichiometric individual reactions, and the appearance of new steady states in different culture stages [6]. In many cases, mechanistic models, based on first principles, are ineffective because of metabolic complexity of microorganisms.

In this sense, complex microbial consortium behavior and culture media with different types of substrates are difficult to model. Nonmechanistic models, or black box models (BBMs), or a combination between mechanistic and nonmechanistic models, or gray box models (GBMs), are more suitable to describe them. Kinetic parameter fitting for WBMs requires experimental measurements of multiple variables, and frequently, model validation may be impractical. BBMs and GBMs constitute alternatives which describe the general dynamic behavior of bioreactors, without requiring many experimental measurements of the system. These models do not offer mechanistic information about metabolic phenomenology present in the system, but they can optimize and control without it. Then, models can be classified based on the mathematical formulation of the system (**Figure 1**). These are classified into mechanistic, empirical, and fermentation models. A mechanistic model is based on deterministic principles. On the other hand, empirical models represent input-output relations without the knowledge of a mechanism. Fermentation process models are usually represented with a combination of both, mechanistic and empirical models.

An important characteristic of modeling is the assumption of homogeneous or heterogeneous conditions. In this sense, a homogeneous system is related to a single continuous phase. In most cases, bioreactors are described as single liquid phases. However, if the biofilm is included in the study, a solid or semisolid phase needs to be considered in the model. On the other hand, heterogeneous systems are related to the description of two or more continuous phases and the interactions between them. Complex heterogeneous systems can be described as multiple phases: liquid,

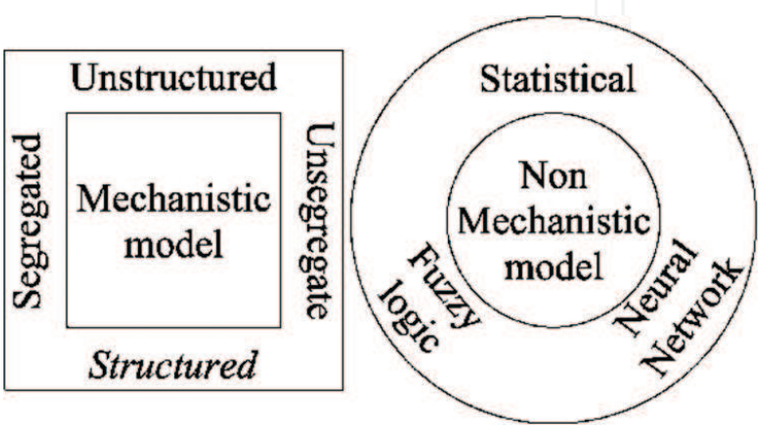

Figure 1.
Classification of models as mechanistic and nonmechanistic.

solid or semisolid, and gaseous phases (e.g. solid-state fermentation). Within this classification, parameters in a model can be classified as distributed and nondistributed (lumped). Distributed parameter models assume that operation parameters vary as a function of space. One, two, or three dimensions are considered in the description of key variables as a function of parameter distribution. As a result, the system is described by a set of partial differential equations (PDEs). On the other hand, a lumped model is necessary, and the system can be described by a set of ordinary differential equations (ODEs), since these parameters do not vary as a function of space.

In this chapter, we provide an overview of mechanistic and empirical models for cell population in fermentation processes.

2. Simple and unstructured kinetic growth models

Unstructured kinetic models (UKMs) represent, in a simple global point of view, the metabolic behavior of the biomass cell production. Mainly, mathematical descriptions for microbial growth kinetics in fermentation processes are based on semiempirical observations. From simple experimental data, we can obtain information to represent cellular growth with unstructured kinetic models.

2.1 Unstructured kinetic models for simple systems

To get the most efficient description of a kinetic model, it is essential to be clear about the application purpose. The application determines the complexity level and structure of the model. The correlation among cell growth, substrate consumption and inhibition [7], or description of the substrate profiles within the reactor during expression of extracellular proteins is the central goal of the model process [8]. The description of key variables is the contribution of the model [9]. These representations are expressed as equations in a simple mathematical model. The UKMs, which are unstructured, unsegregated, are based on the monitoring of cell and nutrient concentration and describe the fermentation process as an average of the species under ideal conditions. Also, it describes the cell and its components as a single species in solution. UKMs consider the apparent rate obtained by metabolic processes, which are carried out by microorganisms. These models are based on conservation equations for cell mass, nutrients, metabolites, and species generation/consumption rates. Most of the UKMs can be divided into three terms: rate expressions for cell growth, rate expressions for nutrient uptake, and rate expressions for metabolite production.

In the case of exponential growth phase, which is the simplest representation of microbial growth, nutrient concentration profiles and decrease rate in several cases are not almost considered.

$$r_X = \frac{dX}{dt} = (\mu - k_D) \cdot X \tag{1}$$

$$r_i = \frac{\alpha}{Y_i}\frac{dX}{dt} \tag{2}$$

where r is the reaction rate, X represents biomass, μ is specific growth rate, k_D is the death rate, α is the stoichiometric factor, and *Yi* is the yield.

The simplest example of multiple reaction models includes substrate consumption for cell maintenance and true yield coefficients (g DCW/g DW) [5]. One of the

most used UKMs is Monod's model [10]. This is one of the simplest models to deal with microbial growth, physiology, and biochemistry. The Monod equation describes the proportional relationship between the specific growth rate and low substrate concentrations (Eq. (3)).

$$\mu = \frac{\mu_{MAX}[S]}{K_S + [S]} \tag{3}$$

where μ_{MAX} is the maximum specific growth rate, [S] is the substrate concentration, and K_S is the saturation constant.

The disadvantage of the model is that the individual entity, regulatory complex, adaptive response to environmental changes, and capacity of cell organelles to generate various products in inherent metabolism cannot be considered. The simplest mathematical models used to estimate microbial growth and substrate consumption are still used for monoclonal antibody production by Chinese hamster ovary (CHO) cells [11, 12]. UKMs can predict specific growth rate in simple systems by calculation of mass balances with independent variables.

2.2 Unstructured kinetic models for a more complicated system

The Monod equation is not able to predict the substrate inhibition effect. Thus, several models including such effects have been developed. For example, Andrew's kinetic equation includes an inhibition function to relate substrate concentration and specific growth rate [13].

$$\mu = \frac{\mu_{MAX}}{1 + K_S/[S] + [S]/K_i} \tag{4}$$

where K_i is the substrate inhibition parameter.

Under the assumption of steady state in continuous operation, substrate concentration is low, and the term $[S]/K_i$ is neglected. Under these conditions, specific growth rate of Andrew's kinetic equation follows Monod equation [13]. Another inhibition function is Aiba's equation for alcoholic fermentation.

$$\mu = \frac{\mu_{MAX}[S]}{K_S + [S]} e^{(-[P]/K_i)} \tag{5}$$

where [P] is the product concentration.

Under the assumption of low product concentration, the term $[P]/K_i \approx 0$, resulting in a simplification to Monod equation [13].

The Monod model assumes that the fermentation culture media has only one limiting substrate. More than one limiting substrate is present and impacts specific growth rate. Thus, the following model considering multiple substrates is proposed [14].

$$\mu = \left(1 + \sum_i^n \frac{[S_{e,i}]}{[S_{e,i}] + K_{e,i}}\right)\left[\prod_j^n \frac{\mu_{\mathrm{MAX},j}[S_j]}{[S_j] + K_{S,j}}\right] \tag{6}$$

where subscript i is the number of each substrate species and e represents the essential substrate.

The limited but accurate information provided by UKMs may help to represent global reactions effectively. In addition to substrate consumption and microbial

growth, fermentations present catabolic inhibition. Therefore, several research groups propose complete UKMs, which include the empirical observation such as variables regarding cells, substrates, and products. Hans and Levenspiel [15] proposed a kinetic model that assumes the existence of inhibitor critical concentrations.

$$\mu = \mu_{MAX}\left[\prod_{i=1}^{h}\left(1-\frac{[I_i]}{[I_i^*]}\right)^{n_i}\right]\left(\frac{[S]}{[S]+K_S\left[\prod_{i=1}^{h}\left(1-\frac{[I_i]}{[I_i^*]}\right)^{m_i}\right]}\right) \tag{7}$$

where [I] is the inhibitor species concentration.

The inhibition function proposed by Levenspiel [16] takes into account the inhibition of ethanol production of alcoholic fermentation modeling, where subscript i corresponds to the substrate or product concentrations. Linear ($n, m = 1$), nonlinear ($n, m > 1$), and fractional ($n, m = 0.5$) applications of these models are possible for fermentation bioreactors (Eq. (7)). An extension of the model was proposed by Luong [7]. He assumes a common mechanism to describe substrate inhibition. Inhibitory factors acting simultaneously could be represented by the following equation:

$$\mu = \mu_{MAX}\left(\frac{[S]}{[S]+K_S}\right)\left(1-\frac{[P]}{[P_{MAX}]}\right)^{n} \tag{8}$$

where [P] is the product species concentration and n is the index of cooperativity between inhibitors.

These models can also explain multiple reactions and include biochemical information of metabolites in the global net effect, making them experimentally accurate. This characteristic is useful for structured and segregated modeling [17]. These models can also describe mixed metabolism [18] and hetero-fermentations [19]. The duality of *Saccharomyces cerevisiae* metabolism, aerobic and anaerobic metabolism, is the best example of multiple reactions. The aerobic growth of the yeast yields biomass by favoring metabolic pathways designed for anabolism and cell division. This metabolism is oxidative in amphibolic reactions. However, at low oxygen concentrations, the yeast metabolism changes from being purely respiratory to partially fermentative. The fermentative pathway mainly leads to ethanol production as a final electron acceptor. Thus, there is a limited growth with high ethanol yields in fermentation culture media. Both metabolisms can occur during the growth of *S. cerevisiae* in a wide range of simple carbohydrate fermentations. At high substrate concentrations, there are limitations in respiratory pathways, which lead to an overflow to ethanol production with enhanced fermentative pathways. The simple WBM with overall reactions could not explain in detail the dualism of both fermentative and respiratory metabolisms. Thus, there are two stoichiometric reactions proposed to explain oxidative and fermentative metabolisms [18].

$$\gamma_1 X + \beta_{11} CO_2 - S - \alpha_{12} O_2 = 0 \tag{9}$$

$$\gamma_2 X + \beta_{21} CO_2 + \beta_{22} P - S = 0 \tag{10}$$

where α, β, and γ are stoichiometric coefficients.

This system considers nearly ideal Monod kinetics, no by-product formation, linear specific oxygen consumption rate, and correlation with substrate uptake. If the primary carbon source is glucose (instead of ethanol), glucose can be used

aerobically and anaerobically. Ethanol can be used as a carbon source only aerobically. Then, different sets of linear algebraic equations can be derived concerning carbon, oxygen, and hydrogen balance.

The respiratory quotient (RQ) is often used as an indicator of fermentative processes. When RQ is close to one, there is no fermentative metabolism, whereas if RQ is above one, the fermentative metabolism occurs.

$$RQ = \left|\frac{r_{CO_2}}{r_{O_2}}\right| = \begin{cases} >1, \textit{fermentative metabolism} \\ \approx 1, \textit{non fermentative metabolism} \end{cases} \tag{11}$$

The mechanistic characteristics of an unstructured, unsegregated kinetic model contribute to the knowledge of the complex metabolism of *S. cerevisiae*. Despite giving relevant information of simple metabolic processes with multiple reactions, UKMs cannot give information about complete intracellular oxidative metabolism. An example of the application of these models is explained in subsequent sections.

3. Structured growth kinetics

There are several classifications of mechanistic and statistical models of cell population for bioprocess applications. Two terms are essential for mathematical description of cell populations: segregated and structured models. A structured model is related to cell material description using multiple chemical components. A segregate model is related to the description of individual cells in a heterogeneous population. Additionally, it is possible to combine a structured approach with a segregated approach. Structured kinetic models are introduced in this section.

3.1 Simple structured kinetic models

Structured kinetic models (SKMs) describe changes in cell population. The liquid phase (abiotic phase) usually contains nutrients for cell growth and some extracellular metabolites. The microorganisms suspended in the liquid phase behave as multicomponent systems. SKMs consider the internal structure of cells (e.g. mitochondria), and the description of cell growth and its metabolism is used to assume a more accurate growth rate. The information used is a starting point to generate schemes that represent more accurately the growth of microorganisms and their cellular components. The complexity of the information variables and parameters increases in SKMs with the mathematical representation of cellular growth.

SKMs are generally classified into morphologically structured models, chemically structured models, genetically structured models, and metabolically structured models [20].

Morphologically structured models consider the kinetics of nutrient consumption and product formation. These models consider different cell types as living species in terms of the role that they play in the overall reaction. Chemically structured models consider the effects of chemical species in fermentation kinetics; all viable cells are functionally similar, and all the fermentation rates and transport phenomena parameters are accounted for. Genetically structured models assume molecular mechanism knowledge. The model includes the rate of expression of an operator-regulated gene and kinetic equations for the transcription, translation, and folding processes. Metabolically structured models provide a better understanding of process regulation mechanisms such as feedback regulation. This model is based on the main metabolic pathways and in most cases is included in MFA. In the

presence of metabolite concentration changes, the network structure represents the reaction and metabolite concentration as a matrix array. Then, SKMs can be classified as dynamic and structural [21]. Dynamic models are described as a set of ordinary differential equations (ODEs). Structural models, which are simplified from ODEs, are represented by a set of algebraic equations through two main approaches: MFA and elementary mode analysis (EMA).

4. Nonmechanistic models

The structured and unstructured kinetic models in the previous sections describe, with a high degree of accuracy, the dynamic behavior of microbial growth in bioreactors. These models, associated with material and energy balances, also help to understand the phenomena associated with microbial metabolism, giving clues to the process design and control.

Black box models (BBMs) usually fall into two main categories: statistical models (SMs) and artificial intelligence tools (AITs). SMs use experimental design, response surface analysis, and exploratory data analysis, whereas AITs consider tools such as data mining, artificial networks, and fuzzy logic [22]. Also, several methodologies to combine mechanistic approaches with nonmechanistic modeling strategies have been developed. The hybrid models, which are known as gray box models (GBMs), inherit the advantages of BBMs such as data analysis and can achieve semi-mechanistic description to each metabolic phenomenon. GBMs offer greater estimation accuracy, calibration ease, better extrapolation properties, and more detailed information on the phenomenology of the system [23]. The advantages of GBMs in the application of bioreactor modeling are direct control and optimization. In this section, we will describe some of these nonmechanistic modeling tools and some of their applications, such as the design of soft sensors.

4.1 Neural network models

Artificial neural networks (ANNs) are mathematical models that are devised from the need to characterize biological neural processes. As the system of ANNs imitates the way which is used to interact with each other in brain neuron, ANNs are simple and strong processes to interconnect the elements that transmit and process information through electrical impulses. In ANNs, these simple process elements are also known as neurons, and depending on the complexity of the connection schemes, they can develop the ability to describe the nonlinear behavior of many dynamic systems [24]. ANNs are computational models that aim to achieve mathematical formalizations of the brain structure and functions, which are constantly reformed by learning through experience and extracting knowledge from the same experience. In ANNs, the hierarchical structure similar to that in brain is established, where neurons connect with each other and transmit the response to other neurons. Once the ANN's structure is defined, it is necessary to develop memory form experience (experimental data). In order to introduce this experience, the ANN training algorithm performs a weight (ω) fitting process associated with each neuron, such that the actions introduced (input signals) converge to the reactions produced (output signal) [24]. Although ANNs do not provide a physical interpretation of the phenomena that take place in the system, these models can approximate the dynamic behavior of the system, making them suitable universal approximators [22]. ANNs are defined based on three basic characteristics: their architecture, activation functions, and training algorithm. The architecture deals with the type of interconnections between their processing units or neurons, while

the activation function corresponds to the dynamic characteristics of the neuron transfer functions. The training algorithm refers to the parameter fitting procedure, which provides the learning ability.

Feedforward networks (FNN) represent the simplest network configuration capable of describing the nonlinear behavior of bioreactors. In FFNs, neurons of each layer propagate their information to all neurons in subsequent layers. In each neuron, the input information corresponds to the weighted sum of all the outputs of the previous layer, and the weighting factors, weights and thresholds, are internally fitted for better system description [24].

Another type of ANNs frequently used is recurrent neural networks (RNNs). The structure of RNNs differ from FFNs, in the sense that some of the last layer neuron output signals are fed back as inputs to any previous layer. RNNs could converge to stable system solutions and include the effects of response delays. These characteristics make these models especially useful in the modeling of continuous bioreactors [25].

4.2 Gray box models

BBMs are based on the analysis of data generated to detect correlations, and basic functionalities between the variables and the WBMs are constructed from first principles. A hybrid model category between WBMs and BBMs is GBMs; these models implement a set of tools that combine some of the characteristics of both. Some of these characteristics include properties of process and control design, without losing the ability to explain the phenomena present in the system. Defining a parallel or serial data flow structure allows the integration of both, mechanistic and nonmechanistic information (e.g., **Figure 2**).

Parallel arrays are mainly used when there is a well-defined mechanistic model of the process and are suitable to improve its estimation performance. It is especially useful in cases where dynamic aspects of the system can be decoupled. **Figure 2A** shows a conceptual diagram of parallel interaction where the circle represents WBMs and the square BBMs, and the circle inside the square corresponds to a hybrid model. In the case of serial arrays, BBMs describe just specific terms of the WBMs, such as growth kinetics or transport parameters. **Figure 2B** represents the hybrid model where the BBMs (square) are substituted into the WBMs (circle). Stosch et al. presented a detailed panorama of this model [23].

In the design of bioreactors and their associated controllers, one of the difficulties is the determination of the kinetic model that adequately describes the growth rate of the respective microorganisms. The selection of a kinetic model leads to restrictive models for fixed operating conditions with little extrapolation possibility.

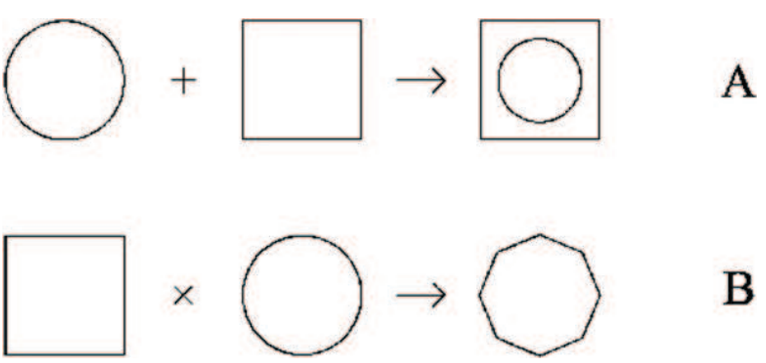

Figure 2.
Classification of models as (A) parallel flow nonmechanistic model and (B) serial flow nonmechanistic model.

GBMs take advantage of the well-known mechanistic information through mass and energy balances to describe specific dynamics of the system and expand its parametric scenario of applicability.

In the determination of bacterial growth kinetic models, GBMs have some advantages compared to BMMs; FFNs are able to describe the system state values, but have important deviations in their estimates, due to their high sensitivity to noise. GBMs combine WBMs with neural network structures, either by feeding the outputs of the ANNs to the state space model, or by backpropagating the estimation error from output layers. Therefore, GBMs offer better forecasting properties and strengthen their performance in the presence of noise [26].

4.3 State observers and soft sensors

Monitoring culture media requires the measurement of variables such as biomass concentration, substrates, dissolved oxygen, carbon dioxide, ammonia, and temperature, among others. These values are used for growth kinetics determination and bioreactor design. However, on multiple occasions, implementation of specific sensors is complicated, and there may be limitations in sensing frequency for variables such as biomass concentration. The implementation of indirect measurement methodologies, such as signal filtering, observer design, and ANNs, allows the estimation of some of these variables, and even the estimation of complex variables such as overall microbial growth rate and the heat flux produced by the system.

In control system theory, a major implementation of state observers is a common complementary strategy. These observers estimate some state variables that cannot be easily measured, either by the absence of suitable sensors, or because of low sampling frequency and high delay times. The main types of observers used for these purposes are those based on the Luenberger scheme, finite-dimensional observers, Bayesian estimators such as Kalman filters, interval observers, observers for fault detection, and even models of artificial intelligence such as ANNs and hybrid models [27].

State observer design requires that the estimated variables are detectable and observable. These states are observable if for a set of specific initial conditions, the internal states of the system are inferred from the knowledge of their outputs. Once its observability is determined, the observer can be designed; the desired type of observer is selected from categories mentioned above. Afterwards, tests of the estimator are carried out by comparing the real values against observer estimates, and in the case of important discrepancies between these values, the observer is adjusted or a different one is selected.

In the case of stirred tank reactors (batch, continuous, or semi-continuous), we may often assume that homogeneous conditions are available, so the system models obtained consist of aggregate parameter systems (ODEs). However, frequently in the case of tubular reactors or solid substrate fermentation systems, homogeneity assumptions are not adequate, so it is necessary to construct using distributed parameter models (PDEs). In the latter case, the design and performance of the main observers, such as Luenberger or Bayesian type, are usually limited, and therefore, it is usual to resort to another type of observer. In such a case, the observers based on a discretized system are substituted for traditional observers [28].

Soft sensors or virtual sensors are used as state observers in specific application. These soft sensors combine several physical measurements with dynamic characteristics to calculate other variables that are not measured.

Soft sensors can not only provide variable information to characterize a system but also facilitate the design of the control schemes.

In bioreactor design, soft sensors can be used to estimate unavailable variables such as biomass. Traditionally, biomass has been traditionally determined by use of a variety of methodologies such as optical density, dry weight, and microbial counts, among others. These techniques present several problems, the most important being the lack of continuous online measurements. To overcome this problem, various strategies have been applied, such as the implementation of low-cost sensors combined with signal processing strategies. For instance, the RGB sensor is used for biomass measurement in microalgae production reactors [29]. This type of sensors uses the intensity of the red, green, and blue (RGB) colors, which correlates with the biomass concentration using dry weight and/or colony formation unit (CFU) information, using the Beer-Lambert law principles. The correlation is described through linear fitting [30]. Additionally, it is possible to compensate background noise by use of ANNs even in the case of nonlinear correlation [31].

Soft sensors can also be applied to nonexplicit system states. These observers can estimate lumped system variables, such as growth rate. As the simplest factor, temperature is commonly used, since it allows estimating system concentrations, due to intrinsic dependence between reaction rates and reaction enthalpy. The heat of reaction, either consumed or dissipated by the system, is one of the implicit system states used for reaction rate determination. The same strategy may also be used to determine microbial growth rates [32].

Microbial growth rates are inherently variable due to their metabolic nature and operation conditions. For example, as fluctuation in substrate concentration occurs in fed-batch bioreactors, the condition of osmotic pressure within cells is modified through the plasma membrane, which may change cellular energetics and the viability of cell division. A suitable strategy for these cases is the design of a substrate consumption rate observer. This kind of observer helps to design a robust control strategy against important fluctuations in maintaining constant substrate concentrations.

The use of observers or soft sensors is an interesting alternative to elucidate approximate values of system states, whether these are explicit or implicit, in cases where online continuous physical measurement is not available. These approximations can be used to design process control schemes that ensure proper functioning.

5. Application examples

There are many practical applications of structured or unstructured kinetic models. In the acetone-butanol-ethanol (ABE) production, the models for the bioprocess have evolved from simple stoichiometric equations to sophisticated and elaborate kinetic models based on metabolic pathways [33, 34], genome-scale metabolic flux modeling [35], system-level modeling [34], and metabolic network [21]. Gordeeva classified mathematical modeling of specific growth rate (dependent or independent on substrate concentration), specific rate of substrate consumption, and specific rate of product formation in batch fermentations [36]. In this study, the states in fermentation are described by a system of three ODEs [36]. Cui reported unstructured lactate formation by enzymatic hydrolysis of sugarcane bagasse, and the model is based on Logistic equations, Luedeking-Piret equations and Luedeking-Piret-like equations [37]. Similarly, Sharma reported an unstructured model to describe growth, substrate utilization, and lactate production by *Lactobacillus plantarum* [38]. On the other hand, the common mathematical descriptions of the fermentation process are based on UKMs. For example, the fermentation of sweet sorghum stalk juice by immobilized *Saccharomyces cerevisiae* is explained by the kinetic parameters of Hinshelwood's model [39]. Another example using the UKM

model is the basic logistic model incorporated with the Luedeking-Piret model (hybrid model) to describe the production of bioethanol from banana and pineapple wastes [40].

Cephalosporium acremonium (ATCC 36225) is one example of the utilization of SKMs where morphological differentiation and catabolite repression are the main aspects of the model approach [41]. SKMs can also effectively represent diauxic growth as well as the monitoring of an intracellular reactant in acetic acid production by *Bacillus licheniformis* [42]. Sansonetti reported a biochemically structured model for ethanol production from ricotta cheese whey by *Kluyveromyces marxianus* [43]. Wang studied a segregated kinetic model in fed-batch culture to represent simultaneous saccharification and co-fermentation (SSCF) for bioethanol production from lignocellulosic raw materials at high substrate concentrations [44]. Another interesting process is the solid-state fermentation. In most proposed models, a set of PDEs is used to describe how intraparticles are diffused or how the growth can be affected by intraparticle diffusion of oxygen, enzymes, hydrolysis products, and other nutrients and the role in the fermentation of other phenomena such as particle shrinkage and spatial microbial biomass distribution [45]. Computational fluid dynamics (CFD) provides information concerning the mixing modeling and design of bioreactors [46]. Another example of CFD is cephalosporin production by *Acremonium chrysogenum;* it was found that the oxygen transfer rate (OTR) directly affects fermentation performance with different impeller combinations [47]. Applications of CFD to fermentation modeling include effects of stress on cell morphology and mass transfer from the bulk solution to the organisms [46]. Biochemical models should be coupled to the CFD models in order to give a closed link between biochemistry and fluid dynamics of the system [33]. Haringa assesses the effect of substrate heterogeneity on the metabolic response of *P. chrysogenum* in industrial bioreactors via coupling of a 9-pool metabolic model with Euler-Lagrange CFD simulations toward rational scale-down and design optimization [48].

Another way to construct mathematical models of microbial growth is the use of FFNs, which describe the behavior of different configurations of bioreactors. An example of this type of applications is the modeling of the production of bioethanol obtained from sugar beets [49]. Here, a three-layer FFN is used to describe the dynamic behavior of the reactor. The first neuron layer consists of system inputs, which correspond to substrate concentration, substrate type, and fermentation time. The second layer corresponds to hidden neurons that process the information through their activation function. Finally, the third layer matches the output of the system that corresponds to the viable cell count of yeasts and the concentration of ethanol produced. On the other hand, GBMs and their hybrid models are not only used to characterize fermentation kinetics but can also describe general behaviors of bioprocesses. For example, in fed batch cultures of *Chlorella pyrenoidosa*, a hybrid scheme of ANN with mass balance mechanistic models describes the general behavior of the states of the system, reducing considerably the variability of their predictions, and achieving versatility in application [50]. These types of GBMs are useful in cases of high complexity due to metabolic dynamics of microorganisms [51]. GBMs or hybrid models are not only combinations of first principles with ANNs, but there may also be hybrid models obtained through the combination of statistical models with ANNs. This type of models usually has special applicability in the optimization of operating conditions of bioreactors (e.g., fed batch fermentation of *Ralstonia eutropha* for poly-β-hydroxybutyrate production) [52].

Soft sensors are also useful in control design. For example, sliding mode observers can describe the behavior of sulfate reduction rate which results from *Desulfovibrio alaskensis* fermentation [53]. These observers use turbidimetric and

colorimetric titration information, and formulate based on sliding modes and sigmoidal functions, but their performance depends strongly on the nature of the system and its monitoring schemes.

Abrupt leaps in substrate concentration can be detected and prevented by the strategy of adaptive or optimal control by coupling with an observation scheme such as ANNs. For example, in L-glutamate production with *Corynebacterium glutamicum* fermentation, physical sensor applications are limited because of high costs and system complexity. However, it is possible to use simpler measurements such as oxygen concentrations, temperature, pH, and carbon dioxide production to train models of ANNs that can approximate the dynamic behavior of glucose concentration [54].

6. Conclusion

In bioprocesses, representation through simple or complex models, fermentation must consider process variables and analytes in mathematical models to achieve optimization, to develop simulations, and to calculate the output of critical variables in bioprocesses. Kinetic models allow predicting the behavior of biochemical reactions. This useful information is critical to techno-economic analysis. The incorporation of simple or complex models could represent phenomena more precisely and thus enhance our comprehension. In the design of bioreactors, a mathematical model is necessary to allow selecting the optimal operating conditions. There is a wide variety of types of models ranging from simple statistical descriptions to artificial intelligence tools. Appropriate model selection depends on the specific application: unstructured models can describe the global behavior, while unstructured models can describe specific phenomena such as metabolic pathways.

Another alternative in the modeling of bioreactors is the black or gray box models, which can be used for bioreactor design, without describing in detail the phenomenology present in the system, which is mainly focused on the global behavior of the system. An important part of the modeling, design, and control of bioreactors is the selection of appropriate sensors. It is often difficult to find suitable sensors for the process, so soft sensors are an interesting alternative to solve this problem.

Once a model describing the dynamical behavior of the bioreactor reaches the available condition, the control scheme can be designed. The goal may be different in each scenario: in the case of variables such as pH, this objective is usually regulation, but in variables such as concentrations and temperature, tracking is usually the goal. In any of these cases, slow and smooth dynamics inherent in these processes usually allow PID controllers to bring system states to the set point efficiently.

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Conflict of interest

The authors declare not to have a conflict of interest.

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References

[1] Stanescu D, Chen-Charpentier B. Random coefficient differential equation models for monod kinetics. Dynamical Systems. 2009:719-728 h ttp://aimsciences.org/journals/pdfs.jsp?paperID=4686&mode=full

[2] Van Impe JF, Poschet F, Geeraerd AH, Vereecken KM. Towards a novel class of predictive microbial growth models. International Journal of Food Microbiology. 2005;**100**:97-105. DOI: 10.1016/j.ijfoodmicro.2004.10.007

[3] Esser DS, Leveau JHJ, Meyer KM. Modeling microbial growth and dynamics. Applied Microbiology and Biotechnology. 2015;**99**:8831-8846. DOI: 10.1007/s00253-015-6877-6

[4] Almquist J, Cvijovic M, Hatzimanikatis V, Nielsen J, Jirstrand M. Kinetic models in industrial biotechnology—Improving cell factory performance. Metabolic Engineering. 2014;**24**:38-60. DOI: 10.1016/j.ymben.2014.03.007

[5] Villadsen J, Nielsen J, Lidén G. Bioreaction Engineering Principles. US: Springer; 2011. DOI: 10.1007/978-1-4419-9688-6

[6] Ferrell JE, Ha SH. Ultrasensitivity part I: Michaelian responses and zero-order ultrasensitivity. Trends in Biochemical Sciences. 2014;**39**:496-503. DOI: 10.1016/j.tibs.2014.08.003

[7] Luong JH. Generalization of monod kinetics for analysis of growth data with substrate inhibition. Biotechnology and Bioengineering. 1987;**29**:242-248. DOI: 10.1002/bit.260290215

[8] Kiefhaber T, Rudolph R, Kohler HH, Buchner J. Protein aggregation in vitro and in vivo: A quantitative model of the kinetic competition between folding and aggregation. Biotechnology (N. Y). 1991;**9**:825-829. DOI: 10.1038/nbt0991-825

[9] Deindoerfer FH. Fermentation kinetics and model processes. Advances in Applied Microbiology. 1960;**2**:321-334. DOI: 10.1016/S0065-2164(08)70134-5

[10] Monod J. The growth of bacterial cultures. Annual Review of Microbiology. 1949;**3**:371-394. DOI: 10.1146/annurev.mi.03.100149.002103

[11] Feisther VA, Ulson De Souza AA, Trigueros DEG, De Mello JMM, De Oliveira D, Guelli Ulson De Souza SMA. Biodegradation kinetics of benzene, toluene and xylene compounds: Microbial growth and evaluation of models. Bioprocess and Biosystems Engineering. 2015;**38**:1233-1241. DOI: 10.1007/s00449-015-1364-0

[12] López-Meza J, Araíz-Hernández D, Carrillo-Cocom LM, López-Pacheco F, Rocha-Pizaña M del R, Alvarez MM. Using simple models to describe the kinetics of growth, glucose consumption, and monoclonal antibody formation in naive and infliximab producer CHO cells. Cytotechnology. 2016;**68**:1287-1300. DOI: 10.1007/s10616-015-9889-2

[13] Andrews JF. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates. Biotechnology and Bioengineering. 1968;**10**:707-723. DOI: 10.1002/bit.260100602

[14] Tsao GT, Hanson TP. Extended Monod equation for batch cultures with multiple exponential phases. Biotechnology and Bioengineering. 1975;**17**:1591-1598. DOI: 10.1002/bit.260171104

[15] Han K, Levenspiel O. Extended monod kinetics for substrate, product,

and cell inhibition. Biotechnology and Bioengineering. 1988;**32**:430-447. DOI: 10.1002/bit.260320404

[16] Levenspiel O. The monod equation: A revisit and a generalization to product inhibition situations. Biotechnology and Bioengineering. 1980;**22**:1671-1687. DOI: 10.1002/bit.260220810

[17] Nielsen J, Villadsen J. Modelling of microbial kinetics. Chemical Engineering Science. 1992;**47**: 4225-4270. DOI: 10.1016/0009-2509 (92)85104-J

[18] Sonnleitner B, Käppeli O. Growth of Saccharomyces cerevisiae is controlled by its limited respiratory capacity: Formulation and verification of a hypothesis. Biotechnology and Bioengineering. 1986;**28**:927-937. DOI: 10.1002/bit.260280620

[19] Tobajas M, Mohedano AF, Casas JA, Rodríguez JJ. Unstructured kinetic model for reuterin and 1,3-propanediol production by *Lactobacillus reuteri* from glycerol/glucose cofermentation. Journal of Chemical Technology and Biotechnology. 2009;**84**:675-680. DOI: 10.1002/jctb.2098

[20] Bapat PM, Bhartiya S, Venkatesh KV, Wangikar PP. Structured kinetic model to represent the utilization of multiple substrates in complex media during rifamycin B fermentation. Biotechnology and Bioengineering. 2006; **93**:779-790. DOI: 10.1002/bit.20767

[21] Millat T, Winzer K. Mathematical modelling of clostridial acetone-butanol-ethanol fermentation. Applied Microbiology and Biotechnology. 2017; **101**:2251-2271. DOI: 10.1007/ s00253-017-8137-4

[22] Sablani S, Datta A, Rahman M, Mujumdar A, Cronin K, Dou J, et al., editors. Handbook of Food and Bioprocess Modeling Techniques. Boca Raton: CRC Press; 2006. DOI: 10.15713/ ins.mmj.3

[23] Von Stosch M, Oliveira R, Peres J, Feyo S, Azevedo D. Hybrid semi-parametric modeling in process systems engineering: Past, present and future. Computers and Chemical Engineering. 2014;**60**:86-101

[24] Haykin S. Neural Network and Learning Machines. Ca: Pearson; 2008. pp. 978-0131471399

[25] Sivakumaran N, Radhakrishnan TK, Babu JSC. Identification and Control of Bioreactor using Recurrent Networks. Instrumentation Science and Technology. 2006;**34**:635-651. DOI: 10.1080/10739140600963871

[26] Acuña G, Cubillos F, Thibault J, Latrille E. Comparison of Methods for Training Grey-Box Neural Network Models. Process Engineering. 2003

[27] Mohd Ali J, Ha Hoang N, Hussain MA, Dochain D. Review and classification of recent observers applied in chemical process systems. Computers and Chemical Engineering. 2015;**76**: 27-41. DOI: 10.1016/j. compchemeng.2015.01.019

[28] Aguilar-Garnica E, García-Sandoval JP, González-Figueredo C. A robust monitoring tool for distributed parameter plug flow reactors. Computers and Chemical Engineering. 2011;**35**:510-518. DOI: 10.1016/j. compchemeng.2010.06.001

[29] Benavides M, Mailier J, Hantson AL, Muñoz G, Vargas A, Van Impe J, et al. Design and test of a low-cost RGB sensor for online measurement of microalgae concentration within a photo-bioreactor. Sensors (Switzerland). 2015;**15**:4766-4780. DOI: 10.3390/s150304766

[30] González-Figueredo C, Aguilar-Garnica E, Guzmán M. Implementación

de un colorímetro webcam para el monitoreo en línea de un sistema de reacción. In: XXXII Encuentro Nac. y 1er Congr. Int. La AMIDIQ; Academia Mexicana de Investigación y Docencia en Ingeniería Química; Riviera Maya, Quintana Roo, México. 2011. pp. 1731-1735

[31] González-Figueredo C, Haro-Córdova F, González-Guerrero FC. Monitoreo de un sistema de agitación utilizando webcam. In: XXXIII Encuentro Nac. y II Congr. Int. AMIDIQ; Academia Mexicana de Investigación y Docencia en Ingeniería Química; San José del Cabo, BCS, México. 2012. pp. 1610-1614

[32] Paulsson D, Gustavsson R, Mandenius CF. A soft sensor for bioprocess control based on sequential filtering of metabolic heat signals. Sensors (Switzerland). 2014;**14**: 17864-17882. DOI: 10.3390/s141017864

[33] Mayank R, Ranjan A, Moholkar VS. Mathematical models of ABE fermentation: Review and analysis. Critical Reviews in Biotechnology. 2013; **33**:419-447. DOI: 10.3109/ 07388551.2012.726208

[34] Chen L, Seung-Oh S, Ting Lu. System-level modeling of acetone-butanol-ethanol fermentation. FEMS Microbiology Letters. 2016;**363**:1-20. DOI: 10.1093/femsle/fnw074

[35] Senger RS, Yen JY, Fong SS. A review of genome-scale metabolic flux modeling of anaerobiosis in biotechnology. Current Opinion in Chemical Engineering. 2014;**6**:33-42. DOI: 10.1016/j.coche.2014.08.003

[36] Gordeeva YL, Rudakovskaya EG, Gordeeva EL, Borodkin AG. Mathematical modeling of biotechnological process of lactic acid production by batch fermentation: A review. Theoretical Foundations of Chemical Engineering. 2017;**51**:282-298. DOI: 10.1134/S0040579517030058

[37] Cui Y, Liu R, Xu L, Zheng W, Sun W. Fermentation kinetics of enzymatic hydrolysis bagasse solutions for producing L-lactic acid. Sugar Tech. 2018;**20**:364-370. DOI: 10.1007/ s12355-018-0592-4

[38] Sharma V, Mishra HN. Unstructured kinetic modeling of growth and lactic acid production by Lactobacillus plantarum NCDC 414 during fermentation of vegetable juices. LWT-Food Science and Technology. 2014;**59**:1123-1128. DOI: 10.1016/j. lwt.2014.05.039

[39] Jin H, Liu R, He Y. Kinetic models and effects of initial total soluble sugar concentrations on batch ethanol fermentation of sweet sorghum stalk juice by *Saccharomyces cerevisiae* strain. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects. 2015;**37**:1282-1290. DOI: 10.1080/ 15567036.2011.616871

[40] Teoh YP, Ooi ZX. Evaluation of unstructured kinetic models for the production of bioethanol from banana and pineapple wastes. BioResources. 2016;**11**:4295-4305. DOI: 10.15376/ biores.11.2.4295-4305

[41] Chu WB, Constantinides A. Modeling, optimization, and computer control of the cephalosporin C fermentation process. Biotechnology and Bioengineering. 1988;**32**:277-288

[42] Larsen R, Kjaergaard L. A structured model for microbial growth and product formation. European Journal of Applied Microbiology and Biotechnology. 1978;**5**: 177-188. DOI: 10.1007/BF00579337

[43] Sansonetti S, Hobley TJ, Calabrò V, Villadsen J, Sin G. A biochemically structured model for ethanol fermentation by *Kluyveromyces marxianus*: A batch fermentation and

kinetic study. Bioresource Technology. 2011;**102**:7513-7520. DOI: 10.1016/j.biortech.2011.05.014

[44] Wang R, Koppram R, Olsson L, Franzén CJ. Kinetic modeling of multi-feed simultaneous saccharification and co-fermentation of pretreated birch to ethanol. Bioresource Technology. 2014; **172**:303-311. DOI: 10.1016/j.biortech.2014.09.028

[45] Rahardjo YSP, Tramper J, Rinzema A. Modeling conversion and transport phenomena in solid-state fermentation: A review and perspectives. Biotechnology Advances. 2006;**24**: 161-179. DOI: 10.1016/j.biotechadv.2005.09.002

[46] Formenti LR, Nørregaard A, Bolic A, Hernandez DQ, Hagemann T, Heins AL, et al. Challenges in industrial fermentation technology research. Biotechnology Journal. 2014;**9**:727-738. DOI: 10.1002/biot.201300236

[47] Duan S, Yuan G, Zhao Y, Ni W, Luo H, Shi Z, et al. Simulation of computational fluid dynamics and comparison of cephalosporin C fermentation performance with different impeller combinations. Korean Journal of Chemical Engineering. 2013;**30**:1097-1104. DOI: 10.1007/s11814-013-0010-2

[48] Haringa C, Tang W, Wang G, Deshmukh AT, van Winden WA, Chu J, et al. Computational fluid dynamics simulation of an industrial *P. chrysogenum* fermentation with a coupled 9-pool metabolic model: Towards rational scale-down and design optimization. Chemical Engineering Science. 2018;**175**:12-24. DOI: 10.1016/j.ces.2017.09.020

[49] Grahovac J, Jokić A, Dodić J, Vučurović D, Dodić S. Modelling and prediction of bioethanol production from intermediates and byproduct of sugar beet processing using neural networks. Renewable Energy. 2016;**85**: 953-958. DOI: 10.1016/j.renene.2015.07.054

[50] Wu Z, Shi X. Optimization for high-density cultivation of heterotrophic *Chlorella* based on a hybrid neural network model. Letters in Applied Microbiology. 2007;**44**:13-18. DOI: 10.1111/j.1472-765X.2006.02038.x

[51] Teixeira A, Cunha AE, Clemente JJ, Moreira JL, Cruz HJ, Alves PM, et al. Modelling and optimization of a recombinant BHK-21 cultivation process using hybrid grey-box systems. Journal of Biotechnology. 2005;**118**:290-303. DOI: 10.1016/j.jbiotec.2005.04.024

[52] Patnaik PR. Neural and hybrid optimizations of the fed-batch synthesis of poly-β-hydroxybutyrate by *Ralstonia eutropha* in a nonideal bioreactor. Bioremediation Journal. 2008;**12**: 117-130. DOI: 10.1080/10889860802261687

[53] Gómez-Acata RV, Neria-González MI, Aguilar-López R. Robust software sensor design for the state estimation in a sulfate-reducing bioreactor. Theoretical Foundations of Chemical Engineering. 2016;**50**:67-75. DOI: 10.1134/S0040579516010036

[54] Ding J, Jia L, Mpofu E, Gao M, Ren X. An on-line adaptive glucose feeding system incorporating patterns recognition for glucose concentration control in glutamate fermentations. Biotechnology and Bioprocess Engineering. 2016;**21**:758-766. DOI: 10.1007/s12257-016-0394-z