Chapter 6

Kinetic Modeling of 1-G Ethanol Fermentations

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Additional information is available at the end of the chapter

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Abstract

The most recent rise in demand for bioethanol, due mainly to economic and environmental issues, has required highly productive and efficient processes. In this sense, mathematical models play an important role in the design, optimization, and control of bioreactors for ethanol production. Such bioreactors are generally modeled by a set of first-order ordinary differential equations, which are derived from mass and energy balances over bioreactors. Complementary equations have also been included to describe fermentation kinetics, based on Monod equation with additional terms accounting for inhibition effects linked to the substrate, products, and biomass. In this chapter, a reasonable number of unstructured kinetic models of 1-G ethanol fermentations have been compiled and reviewed. Segregated models, as regards the physiological state of the biomass (cell viability), have also been reviewed, and it was found that some of the analyzed kinetic models are also applied to the modeling of second-generation ethanol production processes.

Keywords: ethanol fermentation, kinetic modeling, unstructured and unsegregated models, inhibition phenomena, bioreactors

1. Introduction

The interest in producing industrial bioethanol essentially comes from economic and environmental issues. Bioethanol can be produced from batch, fed-batch, and continuous processes, as well as in some cases using flocculating yeasts [1–6].

The development of efficient control strategies for the main operating variables in ethanol fermentations, such as pH, temperature, residual sugars concentration, agitation speed, foam level, among others, requires accurate dynamic models. In addition, mathematical models are important tools for the design, optimization, and control of bioreactors. Bioreactor models seek to describe the overall performance of the bioreactor and consist of two submodels: a balance/

transport submodel that describes mass and heat transfer within and between the various phases of the bioreactor and a kinetic submodel that describes how the rates of the microorganism's growth, substrate consumption, and product formation depend on the key local environmental variables [7].

In ethanol fermentation, the main bioreactions can be summarized by the reductive pathway S $\rightarrow$ X + P + CO_2. According to this reaction, substrates S (glucose and fructose, which result from hydrolysis of sucrose as the limiting substrate), in anaerobic conditions, are metabolized to produce a yeast population X, ethanol P (mainly produced by yeast through the Embden-Meyerhof-Parnas metabolic pathway), and carbon dioxide (CO_2). The hydrolysis of sucrose promoted by the invertase present in the yeast is not the limiting step of ethanol production in industrial processes. The stoichiometry of ethanol-formation reaction from glucose is given by the classical Gay-Lussac equation: $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$

According to Doran [8], both *Saccharomyces cerevisiae* yeast and *Zymomonas mobilis* bacteria produce ethanol from glucose under anaerobic conditions without external electron acceptors. The biomass yield from glucose is 0.11 g/g for yeast and 0.05 g/g for *Z. mobilis*. In both cases, the nitrogen source is NH_3, and the cell compositions are represented by the formula $C_{1.8}O_{0.5}N_{0.2}$. From these data, Doran [9] proposed the following stoichiometric equation for ethanol fermentation (the values of stoichiometric coefficients *a*, *b*, c, *d*, *e*, and *f* are presented in **Table 1**):

$$aC_6H_{12}O_6 + bNH_3 \rightarrow cCH_{1.8}O_{0.5}N_{0.2} + dCO_2 + eH_2O + fC_2H_6O(\text{molar basis})$$

Kinetic modeling of growth, ethanol production, and substrate consumption by yeasts has been traditionally conducted using an unsegregated and unstructured approach for the biomass. This approach ignores the presence of individual cells and structural, functional, and compositional aspects of the cell, describing the complex processes of growth, ethanol production, and substrate consumption through simple kinetic equations [10–15]. **Figure 1** shows a simplified scheme of this approach for the ethanol fermentation process by yeasts and bacteria.

Microorganism	**Stoichiometric coefficients**					
	a	***b***	***c***	***d***	***e***	***f***
Yeast	1	0.16	0.81	1.75	0.35	1.72
Bacteria	1	0.074	0.37	1.89	0.17	1.87

Table 1. Stoichiometric coefficients for ethanol fermentation.

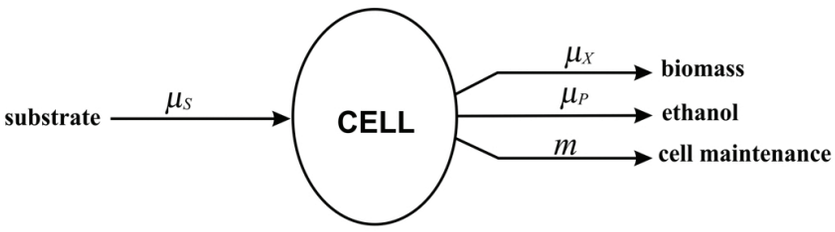

Figure 1. Kinetic modeling of ethanol fermentation based on an unsegregated and unstructured approach for cells (yeasts or bacteria).

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2. Kinetics of cell growth and ethanol formation

In ethanol fermentation, the kinetics of growth and ethanol production are generally the following:

$$\mu_X = f_1(S)g_1(P) \tag{1}$$

$$\mu_p = f_2(S)g_2(P) \tag{2}$$

where μ_X and μ_p, are, respectively, the specific rate of yeast growth and ethanol production, whereas S and P represent the limiting substrate and ethanol concentrations.

2.1. Effect of substrate concentration

The functions $f_1(S)$ *and* $f_2(S)$ are generally of the Monod type [11], except when an inhibition caused by high concentrations of substrate or diffusional limitations occurs due to high cell concentrations.

$$f(S) = \frac{\mu_{\max} S}{K_S + S} \text{(Monod equation)} \tag{3}$$

The inhibition caused by the excess of substrate has generally been modeled by applying the Andrews equation [16–19], though there are other types of equations that are less commonly used [20].

$$f(S) = \frac{\mu_{\max} S}{K_S + S + S^2/K_I} \text{(Andrews equation)} \tag{4}$$

In the case of continuous processes operated near to the steady state, the inhibition concentrations of the substrate are rarely identified. However, inhibitory concentrations can occur during the start-up of these processes or in situations resulting from changes in the substrate feed load.

Atala et al. [21], modeling the effect of temperature upon the kinetics of ethanol fermentation with a high concentration of biomass in a continuous system with total cell retention, used an inhibitory factor (IF) of the exponential type to describe the inhibitory effect of the substrate upon the kinetics of cell growth, which was inserted in the expression of $f(S)$, being $f(S)$, in this case, given by the Monod equation.

$$IF = (e^{-K_I S}) \tag{5}$$

Tsuji et al. [22] evaluating the performance of different ethanol fermentation systems (conventional chemostat, multiple bioreactors, cell recycle bioreactor, extractive bioreactor, and immobilized cell bioreactor) expressed the specific growth rate by an equation analogous to Eq. (1):

$$\mu_X = \mu_1(S)\mu_2(P) \tag{6}$$

One of the analyzed cases considered growth inhibition by substrate, represented by a hyperbolic equation:

$$\mu_1(S) = \frac{\mu_{\max} S}{K_S + S}\left(\frac{K_i}{K_i + S}\right) \tag{7}$$

Sousa and Teixeira [23] reported that one of the main disadvantages of the systems that use flocculating cells (bacteria or yeast) is the reduced reaction rates caused by diffusional limitations of the substrate within the flocs and that, in most cases, the diffusion rate is lower than the reaction rate, which means that the process is controlled by diffusion. Sousa and Teixeira [23] reported that it is generally accepted that yeast flocs are formed by a mediator cation (usually Ca^{2+}) from the interaction between protein and mannans on adjacent cell walls. According to Sousa and Teixeira [23], one means through which to avoid diffusional limitations within the flocs is by using polymeric additives, which act by widening the bridges formed between adjacent cells.

Fontana et al. [24] reported that when the yeast flocs are suspended in a sucrose solution, various phenomena occur simultaneously: the sugar penetrates by diffusion in the aggregates and is hydrolyzed into glucose and fructose by an invertase that is primarily located on the yeast's cell wall. These two sugars diffuse inside and outside of the particle and are fermented in ethanol and CO_2, which in turn diffuse back in the liquid medium.

Fontana et al. [24] assumed that the Fick's law was valid for the aggregate and that the temporal variation of the concentration of each component involved in the transformation is represented by the following equation:

$$\frac{\partial C_i}{\partial t} = D_{ef,i}\frac{\partial^2 C_i}{\partial x^2} + \sum r_i \tag{8}$$

where C_i is the concentration of the component i in the aggregate in time t and distance x as of the floc surface; $D_{\text{ef},i}$ is the effective diffusion coefficient, while $\sum r_i$ is the sum of the consumption or production rates of component i, which are given by Michaelis-Menten-type equations, such as the following:

$$\sum r_i = -r_{S_{\max}}\frac{S}{K_S + S}\ (\text{Sucrose}) \tag{9}$$

$$\sum r_i = +Y_{G/S} r_{S_{\max}}\frac{S}{K_S + S} - r_{G_{\max}}\frac{G}{K_G + G}(\text{Glucose}) \tag{10}$$

where S and G represent, respectively, the concentration of sucrose and glucose, while $Y_{G/S}$ is the conversion factor in glucose based on the hydrolyzed sucrose ($Y_{G/S}$ = 0.505g-glucose/g-sucrose). One relation identical to Eq. (10) can be obtained for fructose.

However, the theoretical descriptions of the diffusional resistances in systems that make use of flocculating microorganisms are generally conducted by introducing a factor into the Monod

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equation that takes into account the reduction in growth rate due to mass transfer limitations. One equation of this type is that proposed by Contois [25].

$$f(S) = \frac{\mu_{\max} S}{K_S X + S} \text{(Contois equation)} \tag{11}$$

According to Menezes et al. [26], the Monod model is appropriate at low cell concentrations, while the Contois model is more appropriate at high concentrations, given that the variable saturation term, $K_S X$, can describe the diffusional limitations present in high cell concentrations. Oliveira et al. [27], modeling a continuous process of ethanol fermentation in a tower bioreactor with recycling of flocculating yeasts, obtained a high value for K_S, which was attributed to the diffusional limitations caused by the high cell concentrations reached in the bioreactor.

2.2. Effect of ethanol concentration

The dependence of μ_X and μ_P on the ethanol concentration is due to the fact that this product has been reported in the literature to act as a noncompetitive inhibitor both for growth and its own production [10, 28–32].

Noncompetitive inhibition is characterized by the fact that in the graph of $1/\mu_X$ or $1/\mu_p$ versus $1/S$ (**Figure 2**), for each ethanol concentration (P), straight lines with different slopes

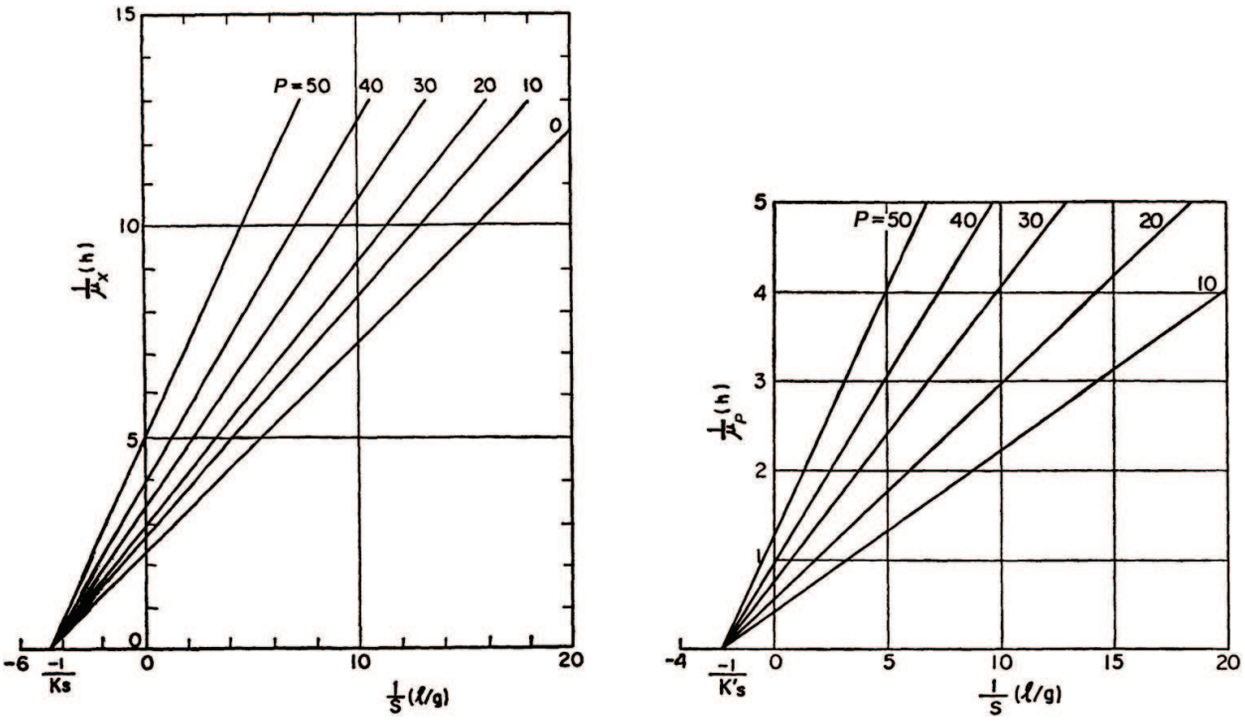

Figure 2. Lineweaver-Burk graph for the specific rates of cell growth (μ_X) and ethanol production (μ_P) (adapted from Aiba et al. [31]).

$\left(\frac{K_{S,i}}{\mu_{max,i}g_i(P)}, \mathrm{i} = 1,2\right)$ and different intercepts $\left(\frac{1}{\mu_{max,i}g_i(P)}, \mathrm{i} = 1,2\right)$ are obtained, but the same intersections with the abscissa are maintained ($-1/K_{S,i}$; $i = 1, 2$).

The molecular base of the mechanism through which the ethanol exerts an inhibitory effect upon fermentation is complex so long as this component, which acts as a denaturing agent, not only acts directly upon the proteins and causes an inactivation or inhibition of the enzymes from the glycolytic pathway but can also act upon the integrity of the lipid membranes, affecting the essential factors, including membrane components, such as transport proteins and the enzymes linked to it [33].

Table 2 presents the main equations proposed for $g_1(P)$ and $g_2(P)$, which are first approximations of much more complicated effects [10, 28–32, 34–42].

Linear (L)	$g(P) = \left(1 - \frac{P}{P_m}\right)$	(12)
Generalized nonlinear (GN)	$g(P) = \left(1 - \frac{P}{P_m}\right)^n$	(13)
Hyperbolic (H)	$g(P) = \left(\frac{K_P}{K_P + P}\right)$	(14)
Parabolic (P)	$g(P) = \left(1 - \frac{P}{P_m}\right)^{0.5}$	(15)
Exponential (E)	$g(P) = (e^{-K_P P})$	(16)

Table 2. Types of commonly proposed equations to describe the inhibitory effect of ethanol upon μ_X and μ_P.

The type of inhibition that affects cell growth is not mandatorily the same as that which affects ethanol production, as it is necessary to determine separately each effect, as proposed by Oliveira et al. [27]. According to Bonomi et al. [17], one of the major difficulties during the development of a mathematical model that fits experimental data of ethanol fermentation is the definition of the type of product inhibition exhibited by the yeast's metabolism. Bonomi et al. [17] reported that this inhibition is characterized by the behavior of the specific growth and production rates with an increase in ethanol concentration, while holding constant the substrate concentration. When developing a mathematical model for a batch system of ethanol production, Bonomi et al. [17] set three values for substrate concentration and determined the corresponding pairs of values (μ_X, P) and (μ_P, P) in each fermentation test. These points—(μ_X, P) and (μ_P, P)—were then plotted to define the types of existing relations between the specific rates and ethanol concentration which, in this case, were both exponential. The values of the specific growth and ethanol production rates were calculated based on experimental data, using the geometric approach proposed by Le Duy and Zajic [43].

The conceptual limitation of the hyperbolic and exponential inhibition is that they predict cell growth and production for all of the ethanol concentrations, even though many experimental tests have shown that cell growth and production cease upon reaching a given high concentration of ethanol [44]. The models of linear, generalized nonlinear, and parabolic inhibition consider that there is a determined concentration of ethanol above which growth and production do not occur. In these models, the P_m parameters represent the ethanol concentrations for which the growth and production processes are completely interrupted.

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In the linear, generalized nonlinear, and parabolic models, the exponents of the term ($1-P/P_m$) are called by Levenspiel [40] as "toxic powers." The values of toxic powers are indicative of how the term of inhibition ($1-P/P_m$) strongly affects the specific growth and ethanol production rates. With the rise in toxic power, the intensity of inhibition increases for a determined ethanol concentration.

In the hyperbolic and exponential inhibition models, the K_P parameters do not admit a physical meaning and can be considered simple empirical constants that apparently depend on the cultivation mode: batch or continuous [31, 32, 37]. Aiba and Shoda [32] argued that the fact that the hyperbolic inhibition constant of the specific growth rate (K_P) has been lower in a batch culture (K_P = 16.0 g/L) than in a continuous culture (K_P =5 5.0 g/L) suggests the possibility that a chemical affinity of ethanol for a key participating enzyme in cell growth appeared in batch experiments. By contrast, the fact that the hyperbolic inhibition constant of the specific ethanol production rate (K'_P) has been lower in continuous cultures (K'_P = 12.5 g/L) than in batch cultures (K'_P = 71.5 g/L) suggests that the ethanol inhibition upon another key enzyme responsible for the fermentation activity was more expressive in continuous experiments.

Another inhibition model commonly used in the literature is that proposed by Luong [37]:

$$g(P) = 1-\left(\frac{P}{P_m}\right)^{\beta} \tag{17}$$

where P_m continues to be the ethanol concentration above which no growth or production can occur, and β is an empirical constant.

One different proposal to describe the inhibitory effects of ethanol upon μ_X and μ_P was presented by Wang and Sheu [45] when they applied multiobjective optimization methods to estimate the parameters of kinetic models of batch and fed-batch processes for ethanol production, using one yeast that is highly tolerant to ethanol (*Saccharomyces diastaticus*). In their study, the kinetic models for the specific rate of cell growth and product formation were represented as follows:

$$\mu_X = \left(\frac{\mu_m S}{K_S + S + S^2/K_{IS}}\right)\left(\frac{K_P}{K_P + P + P^2/K_{IP}}\right) \tag{18}$$

$$\mu_P = \left(\frac{\nu_m S}{K'_S + S + S^2/K'_{IS}}\right)\left(\frac{K'_P}{K'_P + P + P^2/K'_{IP}}\right) \tag{19}$$

Olaoye and Kolawole [46], modeling the kinetics of ethanol fermentation in batch culture of *Kluyveromyces marxianus*, used a semiempirical approach to describe the fermentation process. To model the temporal profile of the biomass concentration, the authors inserted Eq. (20) in the cell mass balance ($dX/dt = \mu_X X$) and analytically integrated the resulting equation to obtain the so-called logistic growth curve (Eq. 21). The ethanol concentration was described, directly applying the modified Gompertz equation (Eq. 22), which represented the empirical part of the proposed mathematical model. The authors did not report the values of the model's parameters.

$$\mu_X = \mu_m\left(1-\frac{X}{X_m}\right) \tag{20}$$

$$X = \frac{X_m}{1+\left(\frac{X_m-X_0}{X_0}\right)e^{-\mu_m t}}; X_0 = X(0) \tag{21}$$

$$P = P_m \exp\left\{-\exp\left[\frac{\mathrm{Pr}_m \exp(1)}{P_m}(\lambda - t)+1\right]\right\} \tag{22}$$

where P_m and Pr_m are, respectively, the maximum concentration and maximum productivity of ethanol; λ is the time of duration of the lag phase, anterior to the exponential phase of ethanol production.

The logistic equation has been used to model fermentation kinetics due to its mathematical simplicity. According to Mitchell [7], the logistic equation can, many times in a single equation, offer an adequate approximation of the entire growth curve, including the lag phase and the cessation of growth in the latter stages of fermentation.

2.3. Effect of cell concentration

The inhibition models presented thus far have been sufficient to satisfactorily describe a large number of fermentations. However, in continuous processes with cell recycling, high cell densities are obtained in the fermenter, and the consideration of other factors, such as the inhibition caused by the excess of biomass, may well be necessary for a better description of the bioprocess kinetic behavior.

The inhibition of cell growth by cell concentrations has been modeled using the following generalized equation [44]:

$$h(X) = \left(1-\frac{X}{X_{\max}}\right)^{\delta} \tag{23}$$

where $X_{\max}$ is the maximum cell concentration that would be reached if ideal conditions for growth were observed, that is, an adequate supply of nutrients and the absence of inhibitory effects [44]. Analogous to the term of inhibition caused by the product, δ indicates the intensity of the inhibition due to the high cell concentrations.

Jarzebski et al. [47], modeling a continuous ethanol fermentation process with high yeast concentrations in a membrane filtration module system, used the following expressions for μ_X and μ_P, in which other formats can be observed for the terms that describe the inhibitory effects of the biomass itself:

$$\mu_X = \left(\frac{\mu_0 S}{K_S+S}\right)\left[1-\left(\frac{P}{P_m}\right)^{A_1}\right]\left[1-\left(\frac{X}{X_m}\right)^{A_2}\right] \tag{24}$$

$$\mu_P = a\exp(-bX) \tag{25}$$

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In cases occurring inhibition of cell growth and product formation by the biomass itself, the expressions of the specific growth and ethanol production rates must be augmented to incorporate these inhibitory effects, that is,

$$\mu_X = f_1(S)g_1(P)h_1(X) \tag{26}$$

$$\mu_P = f_2(S)g_2(P)h_2(X) \tag{27}$$

As regards the procedures of many authors using a kinetic expression for μ_P detached from μ_X, Bu'Lock et al. [10] reported that this does not mean that there is no association between these rates, so long as the ethanol production has been commonly reported in the literature as a process associated with growth. Bu'Lock et al. [10] justified the adoption of such a procedure due to the simplicity and to the better adaptation of such equations to experimental data.

In relating the kinetics of ethanol production to the kinetics of cell growth, the procedure has been to apply the Luedeking-Piret model:

$$\mu_P = \alpha\mu_X + \beta \tag{28}$$

The Luedeking-Piret model is based on the classification of products of the fermentation process as associated ($\alpha > 0$, $\beta = 0$), nonassociated ($\alpha = 0$, $\beta > 0$), and partially associated with cell growth ($\alpha > 0$, $\beta > 0$) [12]. Since ethanol is a product of the primary metabolism of the yeasts, the majority of described cases assumes $\alpha > 0$ and $\beta = 0$, as reported by Oliveira et al. [19] when they modeled the batch ethanol production, using the expression $\mu_P = \alpha\mu_X$, with α = 4.17 g/g. However, it is possible to find descriptions in the literature of ethanol production, parts associated and not associated with cell growth, that is, $\alpha > 0$ and $\beta > 0$, as is the case with that reported by Guidini et al. [18] that used the following equation to describe μ_P in a fed-batch ethanol fermentation process with flocculating yeasts (*S. cerevisiae*):

$$\mu_P = \underbrace{\left(\frac{Y_{P/S}}{Y_{X/S}}\right)}_{\alpha}\mu_X + b \tag{29}$$

Rivera et al. [48], modeling a fed-batch ethanol fermentation process with a strain of industrial yeast (*S. cerevisiae*), used a modified version of the Luedeking and Piret model in which the β coefficient is given as a function of the substrate concentration (S):

$$\mu_P = Y_{P/X}\mu_X + \underbrace{\left(\frac{\beta_m S}{K_{\beta S} + S}\right)}_{\beta} \tag{30}$$

Ghosh and Ramachandran [49], analyzing the effect of *in situ* product removal on the stability and performance of a continuous bioreactor with cell separator for ethanol production, emphasized the use of the Luedeking-Piret model to represent the kinetics of product formation.

In **Table 3**, typical kinetic parameter values for ethanol fermentation are presented [10, 19, 28–32, 34–41].

Table 3 shows large variations in the values of kinetic parameters, demonstrating that these parameters are strongly dependent upon the operational conditions for which they were adjusted, from the culture medium and from the microorganisms used in the fermentation.

Oliveira et al. [50] analyzed the scale-up effects on kinetic parameters and predictions of a mathematical model developed for a continuous process, in small scale, of ethanol fermentation in a tower bioreactor with flocculating yeast recycling, and concluded that the scale-up did not affect the parameter values and that the model continued to be valid to describe the process in the newly investigated scale.

Although the great majority of mathematical models reviewed thus far have been developed for free cell systems, these are equally valid for naturally or artificially immobilized cell systems. However, physiological changes in microbial cells caused by immobilization can significantly affect the values of the kinetic parameters of such models. Moreover, internal and external diffusion effects in microbial particles and flocs can affect the fermentation kinetics. Admassu et al. [38], modeling the hydrodynamics and the profile of product concentration in a tower fermenter for the continuous production of ethanol with flocculating yeasts, reported that the growth and reaction rates for these flocculating microorganisms are frequently limited by mass transfer.

Vicente et al. [51], developing a new technique to measure kinetics and mass transfer parameters in flocs of *S. cerevisiae*, modeled the kinetics of oxygen consumption using the following equation:

	Parameters of $f_1(S)$, $f_2(S)$ $h(X)$	**Parameters of $g_1(P)$ $g_2(P)$**				
		L	**GN**	**P**	**H**	**E**
$\mu_{max,1}$ (/h)	0.11–0.56					
$\mu_{max,2}$ (g/g/h)	0.21–1.90					
$K_{S,1}$ (g/L)	0.07–0.57					
$K_{S,2}$ (g/L)	0.33–60.0					
$P_{m,1}$ (g/L)		87.0–95.0	73.0–87.5	93.6		
$P_{m,2}$ (g/L)		114.0–135.0	87.5	99.0		
n_1 (–)			0.41–4.0			
n_2 (–)			0.41			
$K_{p,1}$ (L/g or g/L)					16.0–105.2	0.016–0.029
$K_{p,2}$ (L/g or g/L)					12.5–71.5	0.015–0.094
X_{max} (g/L)	100.0–330.0					

Table 3. Typical values of kinetics parameters in ethanol fermentation.

$$r_C = -\left(\frac{p_1 C}{p_2 + C}\right) X \tag{31}$$

where r_C is the oxygen consumption rate (mg O_2/(L h)) from which the specific rate of respiration q_O can be calculated, C is the dissolved oxygen concentration (mg O_2/L), X is the active biomass concentration (g/L), p_1 corresponds to $q_{O,m}$ (mg O_2/(g h)), $q_{O,m}$ and p_2 corresponds to K_m (mg O_2/L).

According to Vicente et al. [51], although Eq. (31) represents a Monod-type kinetic model, the calculated values of p_1 and p_2 are only apparent and have no direct relationship with the usual kinetic parameters. Vicente et al. [51] argue that the designations $q_{O,m}$ and K_m were not used because they are generally applied to suspended free cell cultures and that, in this case, cell aggregates were studied, which significantly change the overall behavior of the system and, therefore, the meaning of such parameters.

3. Kinetics of substrate consumption

The kinetics of substrate consumption can generally be described by the Herbert-Pirt model, according to which the substrate is consumed for cell growth and maintenance and the production of a specific product [26]:

$$\mu_S = \frac{\mu_X}{Y^*_{X/S}} + \frac{\mu_P}{Y^*_{P/S}} + m \tag{32}$$

where $Y^*_{X/S}$ and $Y^*_{P/S}$ are, respectively, the stoichiometric coefficients of substrate conversion in cells and product based on the substrate consumed exclusively for each process.

Substrate consumption for cell maintenance refers to the substrate used in the generation of energy for distinct growth functions, such as the maintenance of the concentration gradients between the interior and exterior environment of the cell (osmotic work), synthesis of the cell components that are being continuously degraded, among others [12].

Equation (32) considers that the specific rate of cell maintenance m is a constant, hypothesis, which Ramkrishna et al. [52] do not adopt. According to these authors, the cells suffer a process of degradation in stages in which, at the first stage, the cells would lose their cell viability and, at a second stage, they would die if their maintenance requirements were not attended. To recover the viability, the nonviable cells would need a substrate that would be the same used for growth (exogenous substrate) or an internally stored substrate (endogenous substrate). From these considerations, the following modification in the mathematical representation of the metabolism of maintenance can be introduced, in turn substituting the constant term m in Eq. (32) by a Monod-type expression [26, 53]:

$$\zeta = \frac{\zeta_{\max} S}{K_{S,m} + S} \tag{33}$$

Equation (33) shows that, at high concentrations of substrate, there is a predominance of exogenous metabolism with $\zeta_{max} \rightarrow m$ when $S >> K_{S,m}$, whereas at low concentrations, the endogenous metabolism predominates with $\zeta \rightarrow 0$ when $S \rightarrow 0$.

Generally, the kinetics of substrate consumption is not described using the Herbert-Pirt model to its full extent. The more common approach is the use of apparent coefficients of substrate conversion in cells ($Y_{X/S}$) and ethanol ($Y_{P/S}$), relating μ_S to μ_X or to μ_p by means of these coefficients (Eqs. (34) and (35)). Another approach to describe μ_S is that represented by Eq. (36).

$$\mu_S = \frac{\mu_X}{Y_{X/S}} \tag{34}$$

$$\mu_S = \frac{\mu_P}{Y_{P/S}} \tag{35}$$

$$\mu_S = \frac{\mu_X}{Y_{X/S}} + m \tag{36}$$

Applications of these approaches can be found in the studies listed in **Table 4**.

Sinclair and Kristiansen [12] emphasize the importance of not confusing the stoichiometric coefficients with apparent coefficients as is normally reported in the literature. The stoichiometric coefficient is a constant that depends on the chemical equation, relating the substrates and the products ($Y^*_{P/S}$ = 0.511g-ethanol/g-glucose in the fermentation of glucose to ethanol). The apparent coefficient is the ratio of the mass of a product formed by the total mass of a consumed substrate, which could be participating in multiple reactions, forming a variety of products, including new cells. In this sense, the following definitions for the stoichiometric and apparent coefficients are convenient:

Study	μ_S	Reference
Optimization of an industrial bioprocess of ethanol fermentation with multiple stages and cell recycle, using techniques of factorial design and response surface analysis in combination with phenomenological modeling and simulation	Eq. (34)	[54]
Ethanol fermentation modeling in a tower bioreactor with flocculating yeasts	Eq. (35)	[38]
Analysis of the steady-state stability and modeling of the dynamic behavior of a continuous ethanol fermentation process in a gas-lift tower bioreactor with high cell densities	Eq. (35)	[36]
Bifurcation analysis of two continuous membrane fermentor configurations for ethanol production	Eq. (36)	[55]
Modeling, simulation, and analysis of an ethanol fermentation process with control structure in industrial scale	Eq. (36)	[56]
Modeling of a fed-batch ethanol fermentation process with a strain of industrial yeast (*Saccharomyces cerevisiae*)	Eq. (36)	[48]
Modeling of a fed-batch ethanol fermentation process with flocculating yeasts (*S. cerevisiae*)	Eq. (36)	[18]

Table 4. Mathematical models used for modeling of substrate-consumption kinetics in 1-G ethanol fermentation processes.

$$\bullet Y^{*}_{X/S} = \frac{\text{Mass of new cells formed}}{\text{Substrate mass consumed only for the formation of new cells}} \quad (37)$$

$$\bullet Y_{X/S} = \frac{\text{Mass of new cells formed}}{\text{Total mass of substrate consumed}} \quad (38)$$

$$\bullet Y^{*}_{P/S} = \frac{\text{Mass of product formed}}{\text{Substrate mass consumed only for the formation of product}} \quad (39)$$

$$\bullet Y_{P/S} = \frac{\text{Mass of product formed}}{\text{Total mass of substrate consumed}} \quad (40)$$

Oliveira et al. [57], modeling a continuous ethanol fermentation process in a two-stage tower bioreactor cascade with flocculating yeast recycle, used simplified ($\mu_S = \mu_P/Y_{P/S}$) and generalized ($\mu_S = \mu_X/Y^{*}_{X/S} + \mu_P/Y^{*}_{P/S} + m$) kinetic expressions to describe μ_S and obtained similar predictions of the state variables by both employed approaches.

Bonomi et al. [17], modeling the ethanol production using cassava hydrolyzate in a batch bioreactor, defined the following equation for the mass balance of substrate:

$$\frac{dS}{dt} = -\frac{1}{2}\left(\frac{1}{Y_{X/S}}\mu_X X + \frac{1}{Y_{P/S}}\mu_P X\right) \quad (41)$$

According to these authors, the definition of the apparent coefficients $Y_{X/S}$ and $Y_{P/S}$ guarantee that the terms $\mu_X X/Y_{X/S}$ and $\mu_P X/Y_{P/S}$ are equal; this equality was also reported by Aiba et al. [31] and Ghose and Tyagi [28]. Bonomi et al. [17] argue that the two terms are not exactly equal due to the fact that the calculated values of $Y_{X/S}$ and $Y_{P/S}$ are affected by different experimental errors and the values of μ_X and μ_P are calculated using estimates of other parameters of the model. These authors justify the introduction of the average among the aforementioned terms in Eq. (41), as a means through which to minimize the propagation of errors discussed above.

By contrast, Jin et al. [42], modeling the kinetics of batch fermentation for ethanol production with *S. cerevisiae* immobilized in calcium alginate gel, presented the following mass balance equation for the substrate, without the introduction of the 1/2 factor in the equation:

$$\frac{dS}{dt} = -\left(\frac{1}{Y_{X/S}}\mu_X X + \frac{1}{Y_{P/S}}\mu_P X\right) \quad (42)$$

One equation like $\mu_S = \mu_X/Y_{X/S} + \mu_P/Y_{P/S}$ was also employed by Marginean et al. [58] to model, simulate, and develop proportional integral derivative (PID) control strategies for temperature and the pH of an ethanol production process in a continuous stirred tank reactor (CSTR).

A different proposal was presented by Limtong et al. [35] to model a continuous process of ethanol fermentation in a tower bioreactor with recycle of flocculating yeasts. The authors determined linear relations between the product concentration (P (g/L)) and the specific rates of glucose consumption (μ_S) and ethanol production (μ_P). The ratios between the corresponding angular and linear coefficients of the straight lines (1.63/3.74 and 0.020/0.046)

provide a reasonable estimate of the value of $Y_{P/S}$ (=0.43 g/g), which demonstrates the consistency of such relations (Eqs. (43) and (44)).

$$\mu_S = -0.046P + 3.74(\text{g/g/h}) \tag{43}$$

$$\mu_P = -0.020P + 1.63(\text{g/g/h}) \tag{44}$$

Another situation to be analyzed is when there is more than one fermentable sugar present in the medium, as is the case in the production of beer. Ramirez [59], modeling the dynamic of batch beer fermentation, considered the glucose (G), maltose (M), and maltotriose (N) to be the three majority sugars contained in the fermentative medium. The specific consumption rates of these sugars were described by equations that exhibit a kinetic pattern of preferential use of these substrates, that is, the preferred sugar (G) is first used until its complete exhaustion; next, the second sugar (M), of intermediate preference, is consumed; and lastly, the third sugar (N), the least preferred, is consumed. According to Ramirez [59], this pattern of sequential use is modeled by inserting terms of inhibition of the consumption of a less preferential sugar by one or more preferential sugars in such a way that the specific consumption rates of these sugars μ_i are given by

$$\mu_G = \frac{V_G G}{K_G + G} \tag{45}$$

$$\mu_M = \frac{V_M M}{K_M + M}\left(\frac{K_G^{'}}{K_G^{'} + G}\right) \tag{46}$$

$$\mu_N = \frac{V_N N}{K_N + N}\left(\frac{K_G^{'}}{K_G^{'} + G}\right)\left(\frac{K_M^{'}}{K_M^{'} + M}\right) \tag{47}$$

where V_i is the maximum specific consumption rate of the sugar i (g/g/h), K_i is the saturation constant for the sugar i (g/L), and $K_i^{'}$ is the constant of inhibition caused by the sugar i (g/L).

Additionally, the specific rates of cell growth (μ_X) and ethanol production (μ_E) were given by the following equations [59]:

$$\mu_X = R_{XG}\mu_G + R_{XM}\mu_M + R_{XN}\mu_N \tag{48}$$

$$\mu_E = R_{EG}\mu_G + R_{EM}\mu_M + R_{EN}\mu_N \tag{49}$$

where R_{Xi} and R_{Ei} are, respectively, the stoichiometric yield of the biomass and ethanol per gram of sugar i consumed (g/g).

A similar approach was employed by Lee et al. [60] when they modeled the batch ethanol production by *S. cerevisiae* from a mixture of glucose and maltose. One term ξ was included in the equation of μ_M to represent the glucose repression effect upon the maltose consumption. The final set of the mathematical model equations is presented as follows, highlighting the prediction of diauxic growth in the expression of μ_X and the production of ethanol from the two sugars in the expression of μ_E.

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$$\frac{dX}{dt} = \mu_X X = \left(\frac{\mu_{G,\max} G}{K_G + G} + \frac{\mu_{M,\max} M\xi}{K_M + M}\right)\eta X \tag{50}$$

$$\frac{dG}{dt} = -\mu_G X = -\left(\frac{\mu_{G,\max} G}{Y_{X/G}(K_G + G)}\eta\right)X \tag{51}$$

$$\frac{dM}{dt} = -\mu_M = -\left(\frac{\mu_{M,\max} M\xi}{Y_{X/M}(K_M + M)}\eta\right)X \tag{52}$$

$$\frac{dE}{dt} = \mu_E X = \left(\frac{Y_{E/G}\mu_{G,\max} G}{Y_{X/G}(K_G + G)} + \frac{Y_{E/M}\mu_{M,\max} M\xi}{Y_{X/M}(K_M + M)}\right)\eta X \tag{53}$$

$$\eta = \left(1 - \frac{X}{X_{\max}}\right)\left(1 - \frac{E}{E_{\max}}\right) \tag{54}$$

$$\xi = \frac{1}{1 + G/k_i} \tag{55}$$

4. Loss of cell viability

The loss of cell viability during continuous ethanol fermentation processes with high cell density has been observed by many authors; however, few studies consider this phenomenon in the kinetic modeling of the process.

Jarzebski et al. [47] studied a continuous system of ethanol fermentation consisting of a perfect mixture reactor and a filter with a membrane for separation and posterior recycling of the cells for the fermenter. These authors compared the predictions of an intrinsic model, in which the loss of cell viability was considered, with the predictions of a modified nonintrinsic model where this phenomenon was not considered. The authors concluded that the predictions provided by the two models were similar, and for proposals of simulation and additional analyses of the process, both models could be used. The intrinsic model was thus called because the substrate and ethanol concentrations in this model are defined as regards a corrected volume that neglects the volume occupied by the cells in systems with high cell densities. Monbouquette [61] presented a detailed mathematical development for the formulation of mass balance equations in terms of these intrinsic concentrations.

Lafforgue-Delorme et al. [62] studying a system similar to that of Jarzebski et al. [47] pointed out the need to consider other factors other than the dilution rate and concentration of yeasts that would be important for the modeling of processes with high cell densities, as is the case of continuous ethanol fermentations with cell recycling. These authors developed a model considering the following aspects: dilution and yeast purge, broth viscosity, filter plugging, limitation by substrate, physiological state of the yeasts (cell viability), and inhibition phenomena linked both to ethanol and biomass. They also introduced the concept of steric "stress," according to which, at high cell densities, there would be a reduction in the specific growth rate due to the lack of space for cell division. The effects of inhibition, owing to high cell

concentrations and steric stress, were described, respectively, by the terms $K_X/(K_X + X_V)$ and $(1-X/X_m)$, where K_X is an empirical constant, X_v is the viable cell concentration, and X is the total concentration of cells (viable + nonviable). The final expressions of μ_X and μ_P in the proposed model are given by Eqs. (56) and (57). The predictions of the model agreed satisfactorily with the experimental data both for the operation of the bioreactor with total recycle as well as for the operation with partial recycle.

$$\mu_X = \left(\frac{\mu_{\max} S}{K_S + S}\right)\left(1-\frac{P}{P_m}\right)\left(\frac{K_X}{K_X + X_V}\right)\left(1-\frac{X}{X_m}\right) \tag{56}$$

$$\mu_P = \mu_{Pm} \exp\left(-\frac{K_P X_V}{D}\right) \tag{57}$$

Augusto [63], investigating the influence of the specific rate of oxygen consumption in a continuous ethanol fermentation with high cell density, established the range of 0.1–0.8 mmol O_2/(g-cell h) as being that which the oxygen participates in the metabolism as a micronutrient that is essential to the synthesis of the cell membrane compounds, which would in turn increase the tolerance of the membrane to ethanol and to other inhibitors produced in the fermentation. The greatest tolerance resulted in a lower specific rate of cell death and in a greater efficiency of substrate conversion in ethanol due to the reduction in the value of the maintenance coefficient by the activation of the oxidative catabolic pathway. According to this author, this range of oxygen consumption for which the positive effects of this nutrient are observed in the bioconversion of the substrate would be dependent on the microorganism used, on the fermentation medium, and on the mode in which the process is conducted (batch, fed-batch, and continuous). To calculate the many parameters of fermentation, Augusto [63] segregated the microbial population into viable and nonviable cells, this procedure being possible due to the availability of experimental measures of the concentration of each type of cell separately.

Hojo et al. [41], studying the ethanol production with a strain of flocculating yeast in CSTR with and without cell recycle, concluded that the cell viability was of utmost importance in developing the mathematical model of the process with cell recycle and that cell death is a phenomenon that should be considered in the kinetic modeling of prolonged continuous fermentations in cases in which the hydraulic residence time is high. The kinetic expressions for the specific rates of cell growth (μ_X), substrate consumption (μ_S), ethanol production (μ_P), and cell death (μ_d) were represented by

$$\mu_X = \left(\frac{\mu_{\max} S}{K_S + S}\right)\left(1-\frac{P}{P^*}\right)^n; \mu_{\max} = 0.6/\text{h}^{-1},\ K_S = 0.57\ \text{g/L},\ P^* = 80\ \text{g/L},\ n = 1.8 \tag{58}$$

$$\mu_S = \frac{\mu_X}{Y_g}; Y_g = 0.014\ \text{g/g} \tag{59}$$

$$\mu_P = A + B\mu_S; A = 0.065\ \text{g/g/h}; B = 2.24\ \text{g/g} \tag{60}$$

$$\mu_d = k_d; k_d = 0.0054\ \text{h}^{-1} \tag{61}$$

In the aforementioned works, it was considered that the microbial population consisted solely of viable and nonviable cells, with the latter being incapable of growing and producing the

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desired product. Although inactive in both processes, it was assumed that the nonviable cells remained intact, which means that cell lysis phenomenon was not considered.

For Borzani [64], when intending to apply such an approach, the segregation of the microbial population must be performed considering the active and inactive cells in the growth process, as well as the active and inactive cells in the production process. According to Borzani [64], this differentiation is justified by the fact that a cell that is considered to be active in a given process may not be active in another, or vice-versa. Though quite realistic, this approach is rarely applied, given the enormous experimental difficulty to quantify the concentration of each group of cells separately.

Using an approach that is quite similar to that suggested by Borzani [64], Ghommidh et al. [65], modeling the oscillatory behavior of *Z. mobilis* in continuous cultures for ethanol production, segregated the microbial population in three distinct groups: viable cells that grow and produce ethanol (X_v), nonviable cells that do not grow but produce ethanol (X_{nv}), and dead cells (X_d). The processes of ethanol production, cell growth, loss of viability, and cell death were represented according to the scheme shown in **Figure 3**.

Starting from the scheme proposed by Ghommidh et al. [65], Jarzebski [66] modeled the oscillatory behavior of the state variables X, S, and P in a continuous ethanol fermentation process with *S. cerevisiae*, introducing the concept of combined effect of inhibition by substrate and ethanol simultaneously, since, according to that author, the inhibition by substrate would depend on the ethanol concentration and vice-versa. Taking into account this combined effect of inhibition, Jarzebski [66] proposed the following equations to describe the specific rates of viable cell growth (μ_v), conversion of viable cells into nonviable cells (μ_{nv}), and cell death (μ_d):

$$\mu_v = \left(\frac{\mu_{\max} S}{K_1 + S}\right)\left(1 - \frac{P}{P_c}\frac{S}{K_2 + S}\right) \text{ for } P < P_c(K_2 + S)/S \tag{62}$$

$$\mu_{nv} = \left(\frac{\mu_{\max} S}{K_1 + S}\right)\left(1 - \frac{P}{P_c'}\frac{S}{K_2 + S}\right) - \mu_v \text{ for } P < P_c'(K_2 + S)/S \tag{63}$$

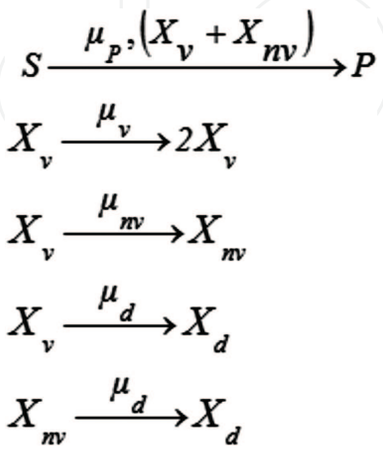

Figure 3. Schematic representation of the cell processes involved in ethanol production by *Zymomonas mobilis* in continuous cultures, according to the model proposed by Ghommidh et al. [65].

$$\mu_d = -\mu_v \text{ for } P < P_c^{'}(K_2 + S)/S \tag{64}$$

Watt et al. [67], using the mathematical model proposed by Jarzebski [66], simulated the continuous ethanol fermentation process for different feed volumetric flow rates and substrate concentrations in the feed stream.

The mathematical modeling of ethanol fermentation processes in which the loss of cell viability occurs is generally conducted by dividing the cell population into two distinct groups: viable cells (X_v) which would be growing and producing ethanol and nonviable or dead cells (X_d), which would be inactive in both processes [68]. The conversion rate from the viable to the nonviable cells is considered to be the first order regarding the concentration of viable cells [12]. The specific rates of cell growth, ethanol production, substrate consumption, and loss of cell viability are defined as regards the viable cell concentration, which refer to the effectively active cells in all of these processes. Mass balance equations for viable and nonviable cells are developed separately. The mass balance equations for ethanol and substrate are similar to those of the conventional model (model that does not incorporate the loss of cell viability) with the previously discussed modifications in the terms involving the specific rates.

Based on these premises, Oliveira et al. [69] developed a mathematical model for a continuous ethanol fermentation process in a tower bioreactor with recycle of flocculating yeasts, in which the loss of cell viability was considered and the predictions of this model were compared with those of the conventional model. Both models provide similar predictions and were equally appropriate for the fermentation process modeling. Later, in another publication, the authors analyzed the scale-up effects on the kinetic parameters and on the predictions of the modified model, and found changes in the values of some of the parameters [70]. In addition, the predictions of the modified model agreed better with the experimental data than did those of the conventional model, especially for the cell concentration variable.

A better description of the fermentation process by the modified model is always the desired result, primarily in those cases in which the levels of cell viability are significantly different than 100%. The cell viability level in ethanol fermentations with high yeast densities has been reported as being strongly dependent on the rate of aeration imposed upon the system [34], varying from 40% to 90% [10, 34, 38, 71]. Under anaerobic conditions, unsaturated fatty acids are not synthesized and the yeasts become more sensitive to ethanol [72]. However, the high levels of cell viability in aerated systems are achieved at the expense of the reduction in ethanol yields [70]. Thus, the rate of aeration is an important variable to be optimized in these systems, seeking to provide an adequate level of oxygen dissolved in the medium [70].

Other aforementioned works in which the segregated approach, regarding cell viability, was applied to describe the microbial population are as follows: Kalil et al. [54], Atala et al. [21], Costa Filho et al. [56], Nelson and Hamzah [53], and Watt et al. [67].

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5. Conclusions

The facility to model the kinetics of ethanol fermentation processes is due to the fact that the governing factors of these processes (limitation by substrate, inhibition, loss of cell viability, death, among others) are well known and that they have a large quantity of mathematical models that have already been developed and made available within the literature.

The present work compiles, in a single publication, a reasonable quantity of kinetic models that are potentially applicable to the adjustment of experimental data of ethanol fermentation processes obtained under the broadest and most varied operating conditions. The models can also be applied to the production processes of another generation, such as is the case of obtaining ethanol from lignocellulosic feedstocks (second-generation bioethanol) for which the literature presents the use of such models as being confirmed by the following recent publications:

- Scott et al. [73]: Attainable region analysis for continuous production of second-generation bioethanol.
- Vásquez et al. [74]: Modeling of a simultaneous saccharification and fermentation process for ethanol production from lignocellulosic wastes by *K. marxianus*.
- Liu et al. [75]: Fermentation Process Modeling with Levenberg-Marquardt Algorithm and Runge-Kutta Method on Ethanol Production by *S. cerevisiae*.

In general, many fermentation studies have confirmed that the unstructured models poorly describe dynamic experiments in which composition and biomass activity change [13, 15]. By contrast, the use of a more detailed approach of cell metabolism, aimed at better describing the dynamic behavior of the process, can lead to the development of structured models containing a large number of variables and parameters. In these cases, the parameter estimation can become a difficult task due to the large experimental effort required and to the need to apply complex numerical methods, which can lead to obtaining parameter values without physical meaning. To illustrate such a scenario, Rivera et al. [76] used a structured model to interpret experimental data of a tower bioreactor for ethanol production by immobilized *S. cerevisiae.* The model contains 34 kinetic parameters and 9 parameters related to the glycolytic and respiratory (tricarboxylic acid [TCA]) pathways. Thus, greater experimental and computational efforts would be required to estimate the parameters associated with this mathematical model.

The class of structured models that are potentially useful is formed by simply applying the structured formulation, through which the description of the quantity and of the biomass properties is performed by using two or three variables, resulting in the so-called two- or three-compartment models. These models combine a better description of the system's behavior with a reasonable mathematical complexity and a smaller number of parameters [77].

Therefore, it is important to balance the complexity of the model with its identification and to seek expressions that are as simple as possible and that are capable of accurately describing the process in both dynamic and steady states [69].

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