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Review Article

Incorporating Inhibition Effects and Hydrolysis Biokinetics into the Mathematical Model of Anaerobic Fermentation

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Abstract

Anaerobic digestion is a well-known biological treatment process. It uses less energy, consumes fewer nutrients, converts organic pollutants into methane gas, and produces a small quantity of biomass. The interactions among the various microbes in this complex biological system need to be better understood, and as a consequence, mathematical models need to be revised. This review discusses the principles of biokinetic models published in the literature on anaerobic fermentation as part of the anaerobic digestion process for waste-activated sludge. Biokinetic models for anaerobic fermentation have been developed to predict cell growth, substrate consumption, and gas production. This exploration delves into the incorporation of the hydrolysis stage, a multi-step process entailing the breakdown of carbohydrates, proteins, and lipids within existing biokinetic models. Because there is no single analytical method for accurately determining the biokinetics of anaerobic fermentation of waste-activated sludge incorporating hydrolysis parameters and inhibition effects are proposed to improve the estimated trends of process variables as a function of the design variables.

Keywords: Anaerobic fermentation; biokinetic; hydrolysis; inhibition; model

1. Introduction

Anaerobic digestion is a widely recognized biological wastewater treatment process that has become the most commonly used method for sludge stabilization (Wang et al., 2021). It is extensively applied to treat various types of wastewater, including manure, domestic, and industrial. This complex and multistep process involves a series of parallel reactions, including a) hydrolysis of complex particulate organic matter, b) fermentation of amino acids and sugars, c) anaerobic oxidation of long-chain fatty acids and alcohols, d) anaerobic oxidation of intermediary products, e) acetate production from carbon dioxide and hydrogen, and f) conversion of acetate to methane (Pavlostathis and Giraldo-Gomez, 1991). Compared to the aerobic process, anaerobic digestion consumes less energy for aeration, requires fewer nutrients, transforms organic contaminants into methane gas, and generates a small amount of biomass (Kumar et al., 2022). Overall, the multifaceted nature of anaerobic digestion, its energy efficiency, and its ability to convert organic waste into valuable methane gas, make it an environmentally-friendly and cost-effective solution for wastewater treatment and sludge stabilization in various industries.

Anaerobic fermentation is a biological process that occurs in the absence of oxygen, wherein organisms carry out their metabolic activities without using oxygen. Anaerobes can thrive in environments with low oxygen levels and have widespread application in critical industrial sectors. However, their involvement in mixed microbial culture processes introduces complexity to the study and modeling efforts (Christy et al., 2014). Anaerobic microbial communities are known to be unstable, exhibiting fluctuations in response to changes in environmental conditions, nutrient availability, and organic loading (Feng et al., 2022 & Yu et al., 2021). These fluctuations can complicate understanding microbial interactions and dynamics in anaerobic fermentation processes. Due to the intricate nature of anaerobic microbial communities and their behaviors, cultivating and manipulating purely anaerobic bacteria necessitate specialized knowledge and rigorous methodologies. Researchers and engineers must consider the complex interactions and factors influencing the performance of anaerobic fermentation to optimize and design efficient processes. In this context, estimating biokinetic information in anaerobic fermentation models becomes challenging. Biokinetics is crucial for understanding the growth rates, substrate utilization, and metabolic activities of microorganisms in the fermentation process. However, enzymes and catabolic pathways involved in fermentation undergo a series of sequential reactions that can be biochemical or physicochemical in origin and occur at varying concentrations (Palanichamy and Palani, 2014). The interactions among the numerous microorganisms in these complex systems are poorly understood, contributing to the lack of comprehensive mathematical models (Feng et al., 2022 & Perendeci et al., 2008).

Biokinetic models play a crucial role in anaerobic fermentation as they are designed to predict essential factors, such as cell growth, substrate consumption, and gas generation during the process (Kurniawan et al., 2018). The model for methanogenesis is relatively straightforward. However, special attention has been dedicated to describing the final step of anaerobic fermentation. This emphasis is particularly crucial since some of the biokinetic models for acidogenesis and acetogenesis have undergone comprehensive review (Zhen et al., 2017). These models are instrumental in understanding the entire anaerobic fermentation process by integrating microbial growth rates with substrate and biomass concentrations. One notable example is the Anaerobic Digestion Model No. 1 (ADM1), where biochemical and physicochemical processes are broken down into multiple equations of biochemical kinetics and mass transfer (Frunzo et al., 2019 Xu et al., 2015). However, the accuracy of data prediction can be influenced by using biokinetic coefficients from previous research. To ensure accurate predictions, each biokinetic variable requires an independent approach, drawing from existing processing simulation results using growth and utilization rate models. By meticulously analyzing and refining the biokinetic models, researchers can acquire valuable insights into anaerobic fermentation processes leading to more effective process optimization and control in diverse applications, including biogas production, wastewater treatment, and sustainable energy generation.

One of the biokinetic models in anaerobic processes is based on the phases of changing complex organic material substances into simple compounds through the processes of hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Bareha et al., 2019 & Batstone, 2006). The hydrolysis model remains an exclusive, rate-determining step, and relatively simplistic model (Batstone, 2006). Hydrolysis biokinetics is more often estimated using first-order biokinetics than microbial growth biokinetics. The hydrolysis stage is a multi-step process that includes the breakdown of carbohydrates, proteins, and lipids. Hence, there is no single analytical method to accurately derive the microbial growth biokinetics as it may also include multiple enzyme production, diffusion, adsorption, reaction, and enzyme deactivation steps (Vavilin et al., 2008). While the first-order biokinetics was not directly related to microbial growth, a high hydrolysis rate showed some real influence on biomass concentration in anaerobic biodegradability experiments using a high inoculum-to-substrate ratio (Bialek, 2012; Vavilin et al., 2008). Consequently, first-order biokinetics is not applicable in all circumstances to estimate accurate hydrolysis parameters.

The current mathematical models of anaerobic fermentation are discussed in this work. It also gives some insight into the development and validation of these mathematical models, which are based on the biokinetics of microbial growth in anaerobic fermentation. The model components, which include the dynamic active cell biomass concentration and dynamic material balance of microbial growth rate expressions, as well as the inclusion of hydrolysis parameters and inhibitory effects, are also discussed in more detail. The inhibitory effects are comprised of two of the most prevalent environmental parameters impacting anaerobic fermentation, pH and temperature, as well as ammonia, which is a significant inhibitory product in anaerobic fermentation.

2. Biokinetics of Microbial Growth

The concept of biokinetics of microbial growth has been dominated by an empirical model initially developed by Monod (Monod, 1949). The Monod equation is widely applied in various fields, including wastewater treatment, bioremediation, and bioprocess engineering, to optimize microbial growth and substrate utilization. It provides valuable insights into how microorganisms respond to changing environmental conditions and helps design efficient biological processes for various applications. The Monod equation introduces a concept that limits the substrate of growth (Monod, 1949) and is defined as following equation (1):

$$\mu = \frac{\mu_{\max} S_h}{K_S + S_h} \tag{1}$$

and is depicted in Figure 1. The parameters μ_{max} and K_S play a fundamental role in the cell-substrate system of microorganisms. The parameter μ_{max} represents the maximum specific growth rate of microorganisms and indicates how fast microorganisms can grow and multiply under optimal conditions (Priyadharshini and Bakthavatsalam, 2019). When the substrate concentration is low, microorganisms can rapidly increase in number, resulting in a high μ_{max} value. However, as the substrate concentration becomes abundant, the growth rate of microorganisms slows down, leading to a lower μ_{max} value (Kythreotou et al., 2014 & Maleki et al., 2018).

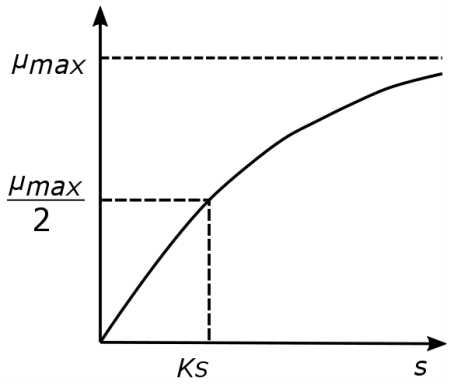

Figure 1. The relationship between the specific growth rate and the concentration of growth-limiting substrate in cell
Source: (Monod, 1949).

On the other hand, the parameter K_S, also known as the half-saturation constant, plays a crucial role as an indicator of the microorganisms' affinity for the substrate. A lower K_S value indicates that microorganisms have a higher affinity for the substrate and can efficiently utilize and consume it even at very low concentrations (Arnaldos et al., 2015 & Maleki et al., 2018). For example, for carbohydrate substrates, K_S values are typically measured in milligrams per liter (mg L^{-1}), while for other substances like amino acids, K_S values are measured in grams per liter (g L^{-1}). This means that microorganisms with

low K_S values need a lower activation energy to thrive on the available substrate (Doran, 2012). In mixed media environments, the growth rates of microorganisms are often much larger than the K_S values for the available substrates. As a result, the microorganisms are not limited by the substrate concentration, and the growth rate has no significant influence on the substrate concentration until the substrate becomes extremely low (S_h becomes extremely low). In such conditions, microorganisms may face substrate limitations, and their growth rates may slow down as the substrate becomes scarce (Gharasoo et al., 2015).

To achieve the μ_{max}, microorganisms require a substrate concentration equal to or greater than the value of K_S. If the substrate concentration is below the K_S value, the microorganisms will not be able to reach their μ_{max}, regardless of the amount of substrate provided (Harmand et al., 2017; Maleki et al., 2018; Qasim and Zhu, 2018). Similarly, when microorganisms are grown at concentrations above the K_S, providing additional substrate will not enhance the μ_{max} further. Therefore, maintaining a substrate concentration in the range of the K_S value is essential to support the optimal growth and performance of microorganisms in biotechnological applications and biological treatment processes. Understanding the K_S value is critical for process optimization and control in practical applications (Qasim and Zhu, 2018). By maintaining the substrate concentration within the K_S range, operators can ensure that microorganisms work at their full potential, improving process performance, reducing treatment time, and higher resource recovery. This significance is fundamental in anaerobic digestion processes where microorganisms are central role in converting organic matter and pollutants into valuable products or harmless byproducts. Furthermore, the K_S value is influenced by various factors, such as temperature, pH, and the presence of inhibitors (Najafpour, 2007 & Rajagopal et al., 2013). Therefore, in designing and operating biological processes, it is essential to consider these factors to maintain the substrate concentration within the optimal range for microorganisms' growth and activity. Additionally, monitoring and controlling the substrate concentration in real time can help ensure the stability and efficiency of the biological processes.

3. Comparison Of Different Microbial Growth Biokinetic Models

The Monod model is a simplified representation of microbial growth kinetics, particularly for microorganisms that exhibit a simple substrate uptake pattern (Maier and Pepper, 2015; Monod, 1949). However, The Monod model is not appropriate for mixed cultures or complicated substrates, and, in some cases, microbial growth may follow a more complex Monod-like response to substrate concentration (Kythreotou et al., 2014). Researchers have developed modified growth rate equations derived from the Monod model to address this limitation and account for more complex growth behavior (Lee et al., 2015 & Mandli and Modak, 2014). These modified equations consider the complexities arising from mixed microbial cultures and diverse substrates (Mandli and Modak, 2014). By incorporating additional parameters and factors, these modified models can better describe and predict microbial growth in various of environments and under different conditions. This adaptability is crucial in various biotechnological applications and wastewater treatment processes, where the composition of microbial communities and the nature of substrates can vary significantly. By exploring and refining these modified growth rate equations, researchers can better understand the factors that influence microbial growth and metabolism. This knowledge can then be applied to optimize and control biological processes more effectively, leading to enhanced performance, higher yields, and improved sustainability in biotechnological applications (Dionisi, 2017).

Several different methods for predicting biodegradation biokinetics that is proportional to the concentration of the growth-limiting substrate are shown in Table 1. These models, including Monod, could predict biokinetic parameters for single anaerobic fermentation and anaerobic fermentation combined with other unit configurations. Tessier model (Eq. 2) for growth represents a more complicated algebraic solution than the Monod rate equation. The growth rate of the Tessier model is susceptible to a low substrate concentration (Najafpour, 2007). The Contois model (Eq. 4) is similar to the Monod rate,

except it has a Michaelis constant proportional to the biomass concentration (X). The Contois model allows for the inclusion of biomass concentration in the growth rate equation, considering the interaction between microorganisms and substrate utilization. This can be particularly useful when studying mixed microbial cultures or complex biological systems. Ming model (Eq. 5) represents the Moser model when the substrate exponential factor is equivalent to a value of 2. Sokol Howell model (Eq. 3) obtains greater values of specific growth rate for the same initial substrate concentration for younger inoculum exposed to lower substrate concentration. Overall, the development of modified growth rate equations from the Monod equation allows for more accurate and comprehensive representations of microbial growth kinetics under various conditions, making them valuable tools in bioprocess modeling, optimization, and control (Wade, 2020).

Table 1. Microbial growth biokinetic models depending on a substrate concentration
Source : (Kythreotou et al., 2014 & Najafpour, 2007).

No	Biokinetic model	Microbial growth rate equation	Equation
1.	Tessier	$\mu = \mu_{\max}\left(1 - e^{-\frac{S_h}{K_S}}\right)$	(2)
2.	Sokol-Howell	$\mu = \frac{\mu_{\max} S_h}{K_S + {S_h}^2}$	(3)
3.	Contois	$\mu = \frac{\mu_{\max} S_h}{K_S X + S_h}$	(4)
4.	Ming	$\mu = \frac{\mu_{\max} {S_h}^2}{K_S + {S_h}^2}$	(5)

Microorganisms' growth and reproduction can be impeded by high substrate and product concentrations (Kythreotou et al., 2014 & Liu, 2012). This inhibition is particularly significant in mixed microbial cultures where suppressing substrates and products can have interconnected effects (11). The original Monod model, which assumes a simple relationship between microbial growth rate and substrate concentration, becomes inadequate when substrates act as barriers to their biodegradation. In such cases, incorporating a correction for substrate inhibition becomes necessary to characterize the growth-related biokinetics accurately. This correction involves introducing an inhibitory constant, K_i, into the Monod derivative (Okpokwasili and Nweke, 2005). By incorporating the inhibitory constant, the modified model accounts for the inhibitory effect of high substrate concentrations on microbial growth (Maleki et al., 2018; Okpokwasili and Nweke, 2005; Xie et al., 2016). The K_i value represents the concentration of substrate at which the growth rate is reduced by half, indicating the threshold beyond which substrate inhibition becomes significant (Wan et al., 2022). With this modification, the model can better describe microbial growth in environments with elevated substrate concentrations, providing a more accurate representation of real-world biological processes.

Product inhibition (P) has comparable repercussions to substrate inhibition. The buildup of end products leads to a steady decline in the rate of specific growth and product synthesis (Mulchandani and Luong, 1989). The growth model expressions must be expanded to encompass product concentrations when inhibitory products compromise cell growth. Table 2 presents various growth biokinetic models that consider both substrate and product inhibition, and these models can be utilized to estimate biokinetic parameters for anaerobic fermentation. The Hinshelwood model (Eq. 6) is a modified version of the Monod model that considers the inhibitory effect of product accumulation on microbial growth. This model allows a more accurate representation of microbial growth in the presence of inhibitory products, providing insights into how the accumulation of certain products can limit microbial activity. The Aiba model (Eq. 7) is another variation of the Monod model that considers the influence of inhibitory products on microbial growth (Aiba et al., 1968). In the Aiba model, the concentration of the product is

incorporated into the denominator of the equation. The Aiba model allows for a more nuanced understanding of how product inhibition affects microbial growth by directly relating the product concentration to the growth rate. The Ghose-Tyagi model (Eq. 8) is a comprehensive growth model considering substrate and product inhibitions in the Monod rate equation (Ghose and Tyagi, 1979). This model accounts for the combined effects of substrate and product concentrations on microbial growth, providing a more accurate representation of the complex interactions between microorganisms and their environment. The Severly model (Eq. 9) is particularly useful when inhibition arises from high concentrations of both substrates and products (Mulchandani and Luong, 1989; Najafpour, 2007). This model is an extension of the Monod equation, considering the squared term of substrate concentration (S^2) in the denominator. It allows for a more detailed examination of how high concentrations of both substrates and products can impede microbial growth.

Table 2. Biokinetic models of microbial growth in relation to substrate and product concentrations
Source: (Aiba et al., 1968; Ghose and Tyagi, 1979; Mulchandani and Luong, 1989; Najafpour, 2007)

No	Biokinetic model	Microbial growth rate equation	Equation
1.	Hinshelwood	$\mu = \left(\frac{\mu_{max} S_h}{K_S + S_h}\right)\left(1 - \frac{P}{P_m}\right)$	(6)
2.	Aiba	$\mu = \left(\frac{\mu_{max} S_h}{K_S + S_h}\right) e^{-K_i P}$	(7)
3.	Ghose-Tyagi	$\mu = \left(\frac{\mu_{max} S_h}{K_S + S_h + \frac{{S_h}^2}{K_i}}\right)\left(1 - \frac{P}{P_m}\right)$	(8)
4.	Severly	$\mu = \left(\frac{\mu_{max} S_h}{K_S + S_h}\right)\left(\frac{K_i}{K_i + P}\right)\left(1 - \frac{P}{P_m}\right)$	(9)

4. Prospective model development on estimating biokinetic parameters for anaerobic fermentation of waste activated sludge

In wastewater modeling, understanding biokinetic parameters is crucial for accurately predicting and optimizing microbial growth and substrate utilization. Biokinetic models characterize microbial behavior and its response to environmental conditions. Several models have been developed to estimate these parameters, considering inhibitory effects and substrate hydrolysis for anaerobic fermentation process. Among the baseline models utilized for estimating biokinetic parameters, Monod, Tessier, Sokol-Howell, Contois, and Ming (Equation 1 – 5) are growth biokinetic models focusing on cell growth rate, substrate uptake, and the influence of inhibitory variables like temperature and pH. These models provide insights into the relationship between microbial growth and substrate concentration, facilitating process optimization and control in various biotechnological applications. On the other hand, Hinshelwood, Aiba, Ghose-Tyagi, and Severly models (Equation 6 – 10) offer a more comprehensive approach by incorporating additional factors such as cell growth rate, substrate consumption, and inhibitor effects. These models consider not only substrate concentration but also the presence of inhibitor compounds like ammonia, which can significantly impact microbial activity. Considering both inhibitory and growth-related factors, these models enable a more accurate depiction of microbial behavior in complex bioprocess environments.

In the complex process of anaerobic fermentation of complex organic compounds, multiple steps are significant in determining the overall process rate. One approach to understanding this process is the concept of the rate-limiting step. The rate-limiting step refers to the particular stage in the fermentation process that becomes the limiting factor for the overall efficiency of the process, mainly under conditions of biokinetic stress (Pavlostathis and Giraldo-Gomez, 1991 & Zhen et al., 2015). In continuous culture systems, biokinetic stress is induced by steadily reducing the solids retention time until it reaches a

critical value, leading to the washout of microorganisms from the system (Pavlostathis and Gossett, 1986). The rate-limiting step can vary depending on factors such as the type of substrate, the specific process design, the operating temperature, and the amount of substrate fed into the system (Mao et al., 2015). Identifying the rate-limiting step is paramount for optimizing the overall efficiency of anaerobic fermentation processes. By understanding which step limits the process rate, researchers and operators can focus their efforts on improving that specific aspect to enhance the overall performance of the fermentation. Furthermore, the concept of the rate-limiting step is essential for troubleshooting and diagnosing potential issues in anaerobic fermentation systems (Batstone et al., 2015; Jimenez et al., 2015; Mao et al., 2015). If the process experiences difficulties or failures, identifying the rate-limiting step can help pinpoint the specific area that needs attention and modification to prevent future failures.

The development of the biokinetic model for anaerobic fermentation considers several critical points the hydrolysis pathways. The interrelationships and overlapping hydrolysis pathways of the complex organic substrates illustrate in Fig. 1. One fundamental aspect is that microorganisms cannot directly utilize complex organic as their growth and methane production sources (Wainaina et al., 2019). Instead, these complex chemicals must undergo hydrolysis, breaking down into assimilable compounds that the microorganisms can readily take up, like carbohydrates, proteins, and lipids (Fig. 1). The hydrolyzed assimilable compounds are not rate-limiting in their transport into the microorganisms and are readily available for microbial uptake (Bamforth and Cook, 2019 & Wainaina et al., 2019). Another important consideration is that a multi-culture complex of macromolecules of

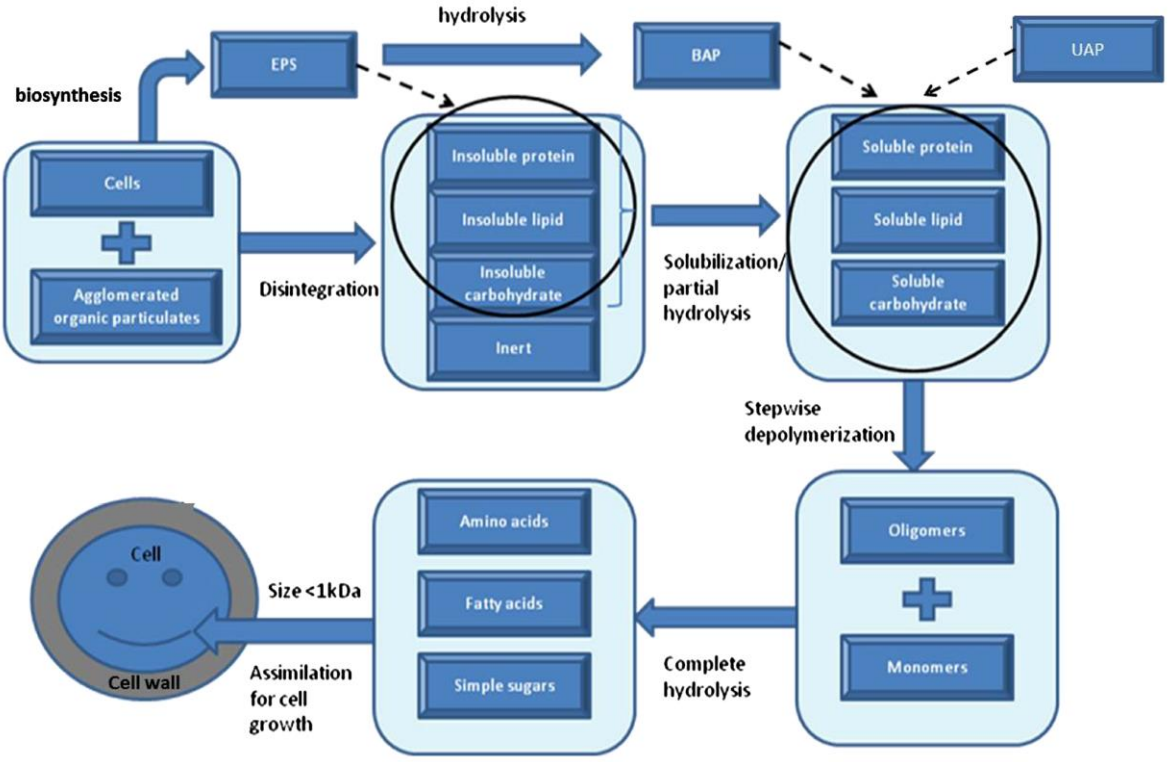

Figure 1. The hydrolysis pathways of EPS, SMP, as well as insoluble and soluble macromolecules
Source: (Teo, 2016)

extracellular polymeric substances (EPS) carrying out the entire anaerobic fermentation process is then solubilized into soluble macromolecules such as soluble microbial products (SMP) (Teo, 2016). This molecule complex comprises a diverse community of microorganisms working collectively as a consortium. The hydrolyzed assimilable compounds serve as the primary substrate for this multi-culture complex, supporting the growth and metabolic activities of the various microbial components (Teo, 2016; Yu et al., 2013). The interactions among the microorganisms within this complex are dynamic and spontaneous, creating a cohesive and functional unit that efficiently carries out the anaerobic

fermentation process. According to the unified theory for EPS and SMP, EPS can be further hydrolyzed into biomass associated products (BAP) component (Fig. 1). Due to the intricate nature of the multi-culture complex and the interactions among its microbial components, the estimation of the biokinetic parameters of anaerobic fermentation has become more comprehensive and complex. This complexity arises from the various factors influencing microbial behavior, such as substrate availability, microbial interactions, and metabolic pathways (Perez-Garcia et al., 2016 & Teo, 2016). As a result, the biokinetic model accounts for a broader range of kinetic constants, reflecting the diverse dynamics and processes occurring within the anaerobic fermentation system.

Anaerobic fermentation is described as a three-step process according to the biokinetic model by the following explanation:

4.1. Extracellular Hydrolysis of Complex Compounds into Soluble Assimilable Substrates

In the first step of anaerobic fermentation, complex organic compounds, such as proteins, lipids, and carbohydrates, are broken down through extracellular hydrolysis. This process involves the secretion of specific enzymes, known as hydrolases, by hydrolytic microorganisms into the surrounding environment. These hydrolases act on the complex compounds, catalyzing their degradation into more straightforward soluble and assimilable substrates (Xu et al., 2014). These soluble substrates are typically smaller molecules, such as amino acids, sugars, and fatty acids, which the microbial community can readily absorb and utilize (Figure 1). A linear trend is used in the hydrolysis process to account for changes in concentration over time in the hydrolysable substrate such that following equation (10):

$$\frac{\mathrm{d}S_\mathrm{h}}{\mathrm{d}t} = K_\mathrm{h}(S_\mathrm{i} - S_\mathrm{h}) \tag{10}$$

The total of the intracellular and extracellular hydrolysis rate coefficients, K_h, in the conceptual model for anaerobic digestion assumes there is no diffusional constraint for transferring solubilized material out of the damaged cell (Barthakur et al., 1991; Kurniawan et al., 2018; Pavlostathis and Giraldo-Gomez, 1991).

4.2. Transport of Soluble Assimilable Substrates into Cells

Before it can be processed by microorganisms, complex organic material must be reduced to a solution that can be transferred across cell membranes (Yu et al., 2013). Transport of a hydrolyzed substrate into the cell is considered directly to the concentration of the active biomass, X, and the difference in concentrations of the hydrolyzed substrate outside and inside the cells. The intracellular concentration of hydrolyzed substrate, S_g, is assumed to be negligible due to the rapid metabolism of the hydrolyzed substrate in the cells. The following relationship can be written as following equation (11):

$$\frac{-\mathrm{d}S_\mathrm{h}}{\mathrm{d}t} = k\left(S_\mathrm{h} - S_\mathrm{g}\right)X = kS_\mathrm{h}X \tag{11}$$

Eqs. (10) and (11) can be rearranged to determine the concentration of hydrolyzed substrate (S_h) by following the equation (12):

$$S_\mathrm{h} = \frac{K_\mathrm{h}\, S_\mathrm{i}}{kX + K_\mathrm{h}} \tag{12}$$

The biomass synthesis yield (X) is defined as the ratio of biomass produced to substrate consumed, as given in the equation (13) below.

$$X = Y(S_{0-\mathrm{i}} - S_\mathrm{i}) + X_0 \tag{13}$$

4.3. Utilization of assimilable substrates for the cell growth and product formation

The Monod biokinetic model has been widely used to describe microbial growth in response to limited substrate concentrations. These model has been instrumental in understanding the relationship between specific growth rates and substrate availability in simple biological systems. However, as wastewater treatment processes involve complex and diverse microbial communities, the applicability of the traditional Monod model becomes limited due to the presence of multiple substrates and competing reactions. To overcome these limitations, researchers have developed unstructured biokinetic models, which provide a more versatile and comprehensive approach to predicting microbial behavior in wastewater treatment processes (Chezeau and Vial, 2019; Noll and Henkel, 2020). These models consider various factors influencing microbial growth and substrate utilization, including the concentrations of different substrates, nutrients, pH, temperature, and inhibitory compounds. Unstructured biokinetic models incorporate multiple rate-limiting steps, account for the interactions between microorganisms and their substrate environment, and offer a more realistic representation of the complex microbial interactions in biological wastewater treatment systems (Chezeau and Vial, 2019 & Moser et al., 2021). The models are usually expressed as differential equations that describe the changes in microbial biomass and substrate concentrations over time. These unstructured growth rate models are dependent on the following:

- the concentration of the substrate (Monod, Tessier, Ming, Sokol-Howell),
- the concentration of the cell and/or the substrate (Contois), and
- the concentration of the substrate and product inhibition (Aiba, Hinshelwood, Ghose-Tyagi, Severly)

The model equations have been interpreted empirically to demonstrate their relevance in modeling anaerobic fermentation biokinetics.

The pH value significantly affects the degradation process during anaerobic fermentation. It directly impacts the microbial community composition and metabolic activities within the anaerobic fermentation reactor. The presence of specific compounds like ammonia, sulfate, and volatile fatty acids (VFAs) can lead to acidity or alkalinity in the system, creating distinct pH conditions that favor the growth of different microbial species (Kythreotou et al., 2014). The pH inhibition factor (I) is a key parameter used to quantify the impact of pH on microbial activity in the system. This factor is essential for understanding how pH affects the performance and stability of the fermentation process. The pH inhibition factor is mathematically expressed as a function that relates the pH level to the microbial growth rate or metabolic rate. The pH inhibition factor (I) is stated as following equation (14) (Batstone et al., 2015):

$$I = \frac{1 + 2 \cdot 10^{0.5(pH_{\min} - pH_{\max})}}{1 + 10^{(pH - pH_{\max})} + 10^{(pH_{\min} - pH)}} \tag{14}$$

Where $pH_{\min}$ and $pH_{\max}$ are two parameters that indicate pH values at which microbial activity is still present. The $pH_{\min}$ and $pH_{\max}$ were 6,5 and 8, respectively, since the pH optimum for most bacteria ranges from pH 3 to 8 (Liu, 2012).

Temperature is a critical element for microbial growth in anaerobic fermentation. The temperature affects microbial activities by altering the nutritional needs, the type of metabolism, the biomass content, and the reaction rate (Delgadillo-Mirquez et al., 2016). Traditionally, the Arrhenius equation has been used to describe the temperature dependency of biological processes. However, in some instances where the Arrhenius equation is unsuitable or applicable to specific parameters, the cardinal temperature model (CTM) is a more appropriate alternative for representing the temperature effect on the anaerobic process (Kythreotou et al., 2014 & Rosso et al., 1993). The CTM considers the experimentally observed inflection point in the suboptimal temperature range. This inflection point is significant as it represents a transition from suboptimal to optimal temperature conditions for microbial growth. The CTM considers this non-linear behavior, providing a more accurate depiction of microbial

response to temperature variations during anaerobic fermentation (Rosso et al., 1993). The CTM temperature factor (θ) is stated as following equation (15):

$$\theta = \frac{(T - T_{\max})(T - T_{\min})^2}{(T_{\text{opt}} - T_{\min})\left[(T_{\text{opt}} - T_{\min})(T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\max})(T_{\text{opt}} + T_{\min} - 2T)\right]} \quad (15)$$

Where T is the operation temperature, $T_{\min}$ and $T_{\max}$ are the lower and upper temperatures when the growth rate does not occur, respectively, and T_{opt} is the temperature at which the maximum specific growth rate equals its optimal value. By using Eqs. (12), (13), (14), and (15), Eqs. (1-9) can be arranged into Eqs. (16-24) to form the biokinetic models on the hydrolyzed substrate in anaerobic fermentation based on microbial growth rate (Table 3). In these biokinetic models, effluent substrate concentration, S_{i}, is a function (dependent) of influent substrate concentration, $S_{\text{i}-0}$.

Table 3. Microbial growth biokinetic models on the hydrolyzed substrate in anaerobic fermentation

Biokinetic model (Eq. no.)	Microbial growth rate on the hydrolyzed substrate
Monod (Eq. 16)	$\frac{\mu_{\max}}{\mu} = \left[\frac{K_{\text{S}}[kY(S_{0-\text{i}} - S_i) + kX_0 + K_{\text{h}}]}{K_{\text{h}}S_{\text{i}}} + 1\right]\left[\frac{1}{f}\right]$
Tessier (Eq. 17)	$\ln\left[1 - \frac{\mu}{\mu_{\max}(f)}\right] = -\left[\frac{K_{\text{h}}S_{\text{i}}}{kK_{\text{S}}[Y(S_{0-\text{i}} - S_{\text{i}}) + X_0] + K_{\text{h}}K_{\text{S}}}\right]$
Sokol-Howell (Eq. 18)	$\frac{\mu_{\max}}{\mu} = \left[\frac{K_{\text{S}}[kY(S_{0-\text{i}} - S_{\text{i}}) + kX_0 + K_{\text{h}}]}{K_{\text{h}}S_{\text{i}}} + \frac{K_{\text{h}}S_{\text{i}}}{kY(S_{0-\text{i}} - S_{\text{i}}) + kX_0 + K_{\text{h}}}\right]\left[\frac{1}{f}\right]$
Contois (Eq. 19)	$\frac{\mu_{\max}}{\mu} = \left[\frac{(K_{\text{S}}Y(S_{0-\text{i}} - S_{\text{i}}) + K_{\text{S}}X_0)(kY(S_{0-\text{i}} - S_{\text{i}}) + kX_0 + K_{\text{h}})}{K_{\text{h}}S_{\text{i}}} + 1\right]\left[\frac{1}{f}\right]$
Ming (Eq. 20)	$\frac{\mu_{\max}}{\mu} = \left[\frac{K_{\text{S}}[kY(S_{0-\text{i}} - S_{\text{i}}) + kX_0 + K_{\text{h}}]^2}{(K_{\text{h}}S_{\text{i}})^2} + 1\right]\left[\frac{1}{f}\right]$
Aiba (Eq. 21)	$\frac{\mu_{\max}}{\mu} = \left[\frac{K_{\text{S}}[kY(S_{0-\text{i}} - S_{\text{i}}) + kX_0 + K_{\text{h}}]}{K_{\text{h}}S} + 1\right]\left[\frac{1}{e^{-k_1 P}}\right]\left[\frac{1}{f}\right]$
Hinshelwood (Eq. 22)	$\frac{\mu_{\max}}{\mu} = \left[\frac{K_{\text{S}}[kY(S_{0-\text{i}} - S_{\text{i}}) + kX_0 + K_{\text{h}}]}{K_{\text{h}}S_{\text{i}}} + 1\right]\left[\frac{-P_m}{P - P_m}\right]\left[\frac{1}{f}\right]$
Ghose-Tyagi (Eq. 23)	$\frac{\mu_{\max}}{\mu} = \left[\frac{K_{\text{S}}[kY(S_{0-\text{i}} - S_{\text{i}}) + kX_0 + K_{\text{h}}]}{K_{\text{h}}S_{\text{i}}} + 1 + \frac{K_{\text{h}}S_{\text{i}}}{K_{\text{i}}[kY(S_{0-\text{i}} - S_{\text{i}}) + kX_0 + K_{\text{h}}]}\right]\left[\frac{-P_{\text{m}}}{P - P_{\text{m}}}\right]\left[\frac{1}{f}\right]$
Severly (Eq. 24)	$\frac{\mu_{\max}}{\mu} = \left[\frac{K_{\text{S}}[kY(S_{0-\text{i}} - S_{\text{i}}) + kX_0 + K_{\text{h}}]}{K_{\text{h}}S_{\text{i}}} + 1\right]\left[\frac{K_{\text{i}} + P}{K_{\text{i}}}\right]\left[\frac{-P_{\text{m}}}{P - P_{\text{m}}}\right]\left[\frac{1}{f}\right]$

Note: f is the inhibition factor as follows: the influence of pH ($f = I$), the influence of temperature ($f = \theta$), the influence of pH-ammonia ($f = I$), the influence of pH-temperature ($f = I\theta$), the influence of temperature-ammonia ($f = \theta$), the

influence pH-temperature-ammonia ($f = I\theta$), the influence of ammonia and no influence of pH-temperature-ammonia (non-f or f is negligible). See Table 2 for selected influence effects.

Monod's and other biokinetic models' mathematical expressions (Table 1 and Table 2) could be changed to include the influence of pH, temperature, and ammonia, resulting in models with combinations of inhibitor factor and product inhibition. Additionally, biokinetic models could be designed without considering the influence of pH, temperature, or ammonia. Both model configurations were compared to anticipate the process's optimal performance since the complexity of microbial activity is cited as a primary cause for the lack of fundamental information about anaerobic fermentation systems (Appels et al., 2011). The effects of pH, temperature, and ammonia on chosen biokinetic models are summarized in Table 4. Monod, Tessier, Sokol-Howell, Contois, and Ming models might be used to estimate biokinetic parameters with and without the impact of pH, temperature, or pH-temperature; and existing biokinetic parameters without the influence of pH, temperature, or pH-temperature. On the other hand, Hinshelwood, Aiba, Ghose-Tyagi, and Severly could be utilized to estimate biokinetic parameters based on ammonia, pH-ammonia, temperature-ammonia, and pH-temperature-ammonia influences.

Table 4. Selected biokinetic models using the influence of pH, temperature, and ammonia.

Effect	Variable input	Biokinetic model								
		MO[a]	TE[b]	SH[c]	CO[d]	MI[e]	HI[f]	AI[g]	GT[h]	SE[i]
pH	I	•	•	•	•	•				
Ammonia	P, P_m						•	•	•	•
Temperature	θ	•	•	•	•	•				
pH-ammonia	I, P, P_m						•	•	•	•
pH-temperature	I, θ	•	•	•	•	•				
Temperature-ammonia	θ, P, P_m						•	•	•	•
pH-temperature-ammonia	I, θ, P, P_m						•	•	•	•
No pH-temperature-ammonia	-	•	•	•	•	•				

[a]*Monod;* [b]*Tessier;* [c]*Sokol-Howell;* [d]*Contois;* [e]*Ming;* [f]*Hinshelwood;* [g]*Aiba;* [h]*Ghose-Tyagi;* [I]*Severly*

Through rigorous statistical analysis, the selected biokinetic models possess a high degree of accuracy in predicting the behavior of anaerobic fermentation reactors (Maleki et al., 2018). These biokinetic models have undergone extensive testing and validation to assess their predictive capabilities under varying conditions and scenarios. The statistical analysis involved comparing model-predicted data with actual experimental results obtained from anaerobic fermentation reactors. To evaluate the performance of the biokinetic models, various statistical metrics and error measures were employed, including the coefficient of determination (R^2), root mean square error (RMSE), mean absolute error (MAE), and relative percentage error (RPE) (Alavi and Ansari, 2022; Yaqub and Lee, 2022; Zhong et al., 2021). These metrics allowed researchers to quantitatively assess the agreement between the model predictions and the experimental data. A high R^2 value close to 1 indicates a strong correlation between the model predictions and the actual measurements, signifying the model's ability to capture the underlying processes accurately. Moreover, low RMSE and MAE values indicate that the model's predictions are close to the observed data points, indicating high accuracy. Additionally, the RPE provides insights into the percentage deviation between the model predictions and the experimental values, enabling a comprehensive assessment of model performance (Huang et al., 2020).

Additionally, the models' complexity and goodness of fit were evaluated using Bayesian Information Criteria (BIC) and Akaike Information Criteria (AIC) (Kurniawan et al., 2018). The BIC and AIC allowed for a comprehensive comparison of the models' abilities to balance the trade-off between complexity and accuracy, aiding in selecting the most appropriate model. Furthermore, bias and accuracy factors were utilized to gauge the models' ability to capture the dynamic behavior of anaerobic fermentation processes and account for any systematic deviations between model predictions and experimental data (Kurniawan et al., 2018 & Tao et al., 2014). These factors allowed for a thorough assessment of the models' reliability in representing the intricate microbial interactions and metabolic pathways involved in anaerobic fermentation.

5. Conclusions

The microbial growth models may be changed by adding the hydrolysis parameters and inhibitory effects to offer a full update for the anaerobic fermentation process. An organism's half-saturation constant, a biokinetic parameter known as a hydrolyzed substrate transport rate coefficient, and a biochemical yield rate coefficient are all examples of biokinetic parameters. These approaches might be interesting to a large scientific community addressing anaerobic biological wastewater treatment, mathematical modeling, simulation, and optimization of the process. Further investigation is warranted for the empirical and mechanistic validation of these proposed update models.

6. Abbreviations

f	Inhibition factor
I	pH factor
k	Hydrolyzed substrate transport rate coefficient (L g^{-1} d^{-1})
K_h	Substrate hydrolysis rate coefficient (d^{-1})
K_i	Inhibitory constant required to produce half maximum inhibition (g L^{-1})
K_S	Half-saturation constant with respect to hydrolyzed substrate (g L^{-1})
P	Product inhibition concentration (g L−1)
P_m	Maximum product inhibition concentration (g L^{-1})
pH_{max}	pH maximum for most bacteria ranges
pH_{min}	pH minimum for most bacteria ranges
S_g	Concentration of hydrolyzed substrate intracellular cell (g L^{-1})
S_h	Concentration of hydrolyzed substrate (g L^{-1})
S_{o-i}	Substrate concentration in the influent (g L^{-1})
S_i	Substrate concentration in the effluent (g L^{-1})
T	The operation temperature (˚C)
T_{max}	The upper temperature (˚C)
T_{min}	The lower temperature (˚C)
T_{opt}	The temperature at which the maximum specific growth rate equals its optimal value (˚C)
X	Concentration of active cell biomass (g L^{-1})
X_o	Initial concentration of active cell biomass (g L^{-1})
Y	Biomass yield coefficient (g g^{-1})
μ	Maximum specific growth rate of a microorganism (d^{-1})
μ_{max}	Maximum specific growth rate of a microorganism (d^{-1})
θ	Temperature factor

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