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Incorporating the inhibition effect and hydrolysis kinetics into the mathematical model of waste activated sludge 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 are poorly understood, and as a consequence, mathematical models are inadequate. 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 throughout the process. We explore how the hydrolysis stage, which is a multi-step process that involves the breakdown of carbohydrates, proteins, and lipids, may be included in current biokinetic models. Because there is no single analytical method for accurately determining the biokinetics of anaerobic fermentation of waste activated sludge, the incorporation of 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, hydrolysis, inhibition, waste activated sludge

Abbreviation

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})
$S_{0\text{-}i}$	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_0	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

1. Introduction

Anaerobic digestion is a well-known biological wastewater treatment process that has evolved into the most often used method of sludge stabilization. Being commonly utilized to treat manure, domestic wastewater, and industrial wastewater [1], this complex and multistep process consists of series and parallel reactions such as 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 [2]. In comparison 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 [3].

Anaerobic fermentation is an anaerobic process that depicts an organism's active metabolism without oxygen. While anaerobes may thrive in less-than-optimal conditions and have been exploited in a range of key industrial sectors, their role in mixed microbial culture processes complicates the study and modeling [4]. Anaerobic microbial communities are often unstable, fluctuating in response to environmental changes,

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nutrient availability, and organic loading [4,5]. Consequently, the culture and manipulation of purely anaerobic bacteria require specialized knowledge and rigorous methodologies [4].

It has been difficult to properly estimate the available biokinetic information in the anaerobic fermentation model, which is used as a logical foundation for process analysis, control, and design. Enzymes and catabolic pathways undergo several sequential reactions during fermentation, which may be biochemical or physicochemical in origin and with varying concentrations [6]. The interactions among the many microorganisms in these complex systems are not well understood, and as a result, mathematical models are lacking [5].

Biokinetic models for anaerobic fermentation have been created to predict cell growth, substrate consumption, and gas generation in the process. In addition to the straightforward model of methanogenesis, special attention was given to descriptions of the final step of anaerobic fermentation, despite the fact that some of the biokinetics models of acidogenesis and acetogenesis have been thoroughly reviewed [1]. These models help to understand the entire process by combining microbial growth rates with substrate and biomass concentrations. In the complex of Anaerobic Digestion Model No. 1 (ADM1), biochemical processes and physicochemical processes are decomposed into multi-equations of biochemical kinetics and mass transfer. Several kinetic coefficients are taken from previous research which affects the accuracy of data prediction. Thus, each biokinetic variable requires an independent approach based on the existing processing simulation results by using simple growth kinetic models and utilization rate.

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. The hydrolysis model remains an exclusive, rate-determining step, and relatively simplistic model [7]. 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 [8]. 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 [8,9]. Consequently, the 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 [10]. The Monod equation introduces a concept that limits the substrate of growth as follows, $\mu = \frac{\mu_{max} S_h}{K_S + S_h}$, and is depicted in Figure 1. The μ_{max} and K_S parameters are fundamental to the cell-substrate system. The μ increases rapidly in the presence of low substrate concentrations and slowly in the presence of large substrate concentrations [11,12]. K_S values are typically extremely low, measured in mg L^{-1} for carbohydrate substrates and in g L^{-1} for other substances like amino acids [13]. The growth rates that constrain the substrate in mixed media are often much larger than K_S . As a result, the growth rate has no influence on the substrate concentration until S_h becomes extremely low.

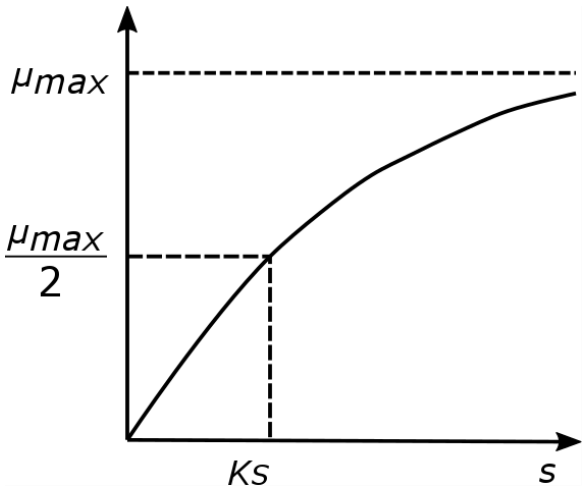

Figure 1. The relationship between the specific growth rate and the concentration of growth-limiting substrate in cell culture [10].

K_S is a factor that influences a cell's affinity for a substrate. If microbial cells exhibit a high affinity, as indicated by a low K_S value, they also need a low activation energy to thrive. When microorganisms are grown at concentrations below the K_S, the μ_{max} will not be reached. When microorganisms are grown at concentrations above the K_S, any substrate provided will not augment the μ_{max} . As a result, the microorganisms' growth substrate should be equal to or greater than the value of K_S.

3. Comparison of different microbial growth biokinetic model

The Monod model is accurate when applied with pure cultures and simple substrates [10]. This model, however, is not appropriate for mixed cultures or complicated substrates [11]. However, 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, which include Monod [10], could be used to predict biokinetic parameters for both single anaerobic fermentation and anaerobic fermentation combined with other unit configurations. Tessier model (Eq. 1) for growth represent a more complicated algebraic solution than the Monod rate equation. The growth rate of Tessier model is very sensitive to a low substrate concentration [15]. The Contois model (Eq. 3) is somehow similar to the Monod rate, except it has a Michaelis constant that is proportional to the biomass concentration (X). Ming model (Eq. 4) represents the Moser model when the substrate exponential factor is equivalent to a value of 2. Sokol Howell model (Eq. 2) obtains for the same initial substrate concentration, greater values of specific growth rate for younger inoculum exposed to lower substrate concentration.

Table 1. Microbial growth biokinetic models depending on a substrate concentration [11,14,15].

Biokinetic model	Microbial growth rate equation	
Tessier	$\mu = \mu_{max}\left(1 - e^{-\frac{S_h}{K_S}}\right)$	(1)
Sokol-Howell	$\mu = \frac{\mu_{max} S_h}{K_S + {S_h}^2}$	(2)
Contois	$\mu = \frac{\mu_{max} S_h}{K_S X + S_h}$	(3)
Ming	$\mu = \frac{\mu_{max} {S_h}^2}{K_S + {S_h}^2}$	(4)

Microorganisms' growth and reproduction can be impeded by high substrate and product concentrations [11,16]. In mixed microbial cultures, suppression of both products and substrates may have comparable effects and are interrelated [11]. When a substrate acts as a barrier to its own biodegradation, the original Monod model becomes particularly undesirable. In this situation, a correction for substrate inhibition may be incorporated into the Monod derivative by including an inhibitory constant, K_i, to accurately characterize the growth-related biokinetics [17].

Product inhibition 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 [16]. The growth model expressions must be expanded to encompass product concentrations P when the presence of inhibitory products compromises cell growth. Table 2 shows the growth biokinetic models that are based on the concentration of the substrate and the inhibition of the product. These models could be used to estimate biokinetic parameters for anaerobic fermentation. Hinshelwood model (Eq. 5) describes Monod rate correcting with product inhibition, while Aiba model (Eq. 6) relates to the term Monod rate correction which is applied using the concentration of the product. Ghose-Tyagi model (Eg. 7) gives the description of substrate and product inhibitions incorporating in Monod rate. Moreover, inhibition may be due to high product and substrate concentrations, the following model maybe useful for rate equation, which is very close to the Severly model (Eq. 8).

Table 2. Biokinetic models of microbial growth in relation to substrate and product concentrations [15,16,18,19].

Biokinetic model	Microbial growth rate equation	
Hinshelwood	$\mu = \left(\frac{\mu_{max} S_h}{K_S + S_h}\right)\left(1 - \frac{P}{P_m}\right)$	(5)
Aiba	$\mu = \left(\frac{\mu_{max} S_h}{K_S + S_h}\right) e^{-K_i P}$	(6)
Ghose-Tyagi	$\mu = \left(\frac{\mu_{max} S_h}{K_S + S_h + \frac{{S_h}^2}{K_i}}\right)\left(1 - \frac{P}{P_m}\right)$	(7)
Severly	$\mu = \left(\frac{\mu_{max} S_h}{K_S + S_h}\right)\left(\frac{K_i}{K_i + P}\right)\left(1 - \frac{P}{P_m}\right)$	(8)

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

Biokinetic parameters may be developed by including inhibitory effects and hydrolysis parameters on hydrolyzing substrates. To estimate biokinetic parameters, the Monod, Tessier, Sokol-Howell, Contois, Ming, Hinshelwood, Aiba, Ghose-Tyagi, and Severly models might be used as baseline models. Monod, Tessier, Sokol-Howell, Contois, and Ming are growth biokinetic models that incorporate parameters for cell growth rate, substrate uptake, and inhibitory variables such as temperature and pH. On the other hand, Hinshelwood, Aiba, Ghose-Tyagi, and Severly are growth biokinetic models that take into consideration characteristics such as cell growth rate, substrate consumption, inhibitor factors, and inhibitor products such as ammonia.

Several steps are involved in the anaerobic fermentation of complex organics. The rate-limiting step approach may be used to explain the anaerobic fermentation process in general. The rate-limiting step is defined as the step that will lead to process failure in the presence of biokinetic stress [2]. In the context of continuous culture, biokinetic stress refers to the application of a steadily decreasing solids retention time until it falls below its limiting value, resulting in microorganism washout [20]. During anaerobic fermentation, the rate-limiting phase in the overall process is dependent on the type of the substrate, the process design, the temperature, and the amount of substrate being fed into the system [21].

The following points were taken into account in the development of the biokinetic model [22]. The microbes cannot gather complex chemicals without first hydrolyzing them into assimilable compounds, which is the primary source of growth and methane production for the

microorganisms. Hydrolyzed assimilable compounds are not rate-limiting in their transport into microorganisms. The entire process of anaerobic fermentation is carried out by a multi-culture complex that uses hydrolyzed assimilable compounds. The microbial components of this multi-culture complex communicate and behave spontaneously as a unit. Given these assumptions, the model's kinetic constants are much broader in scope and are complex.

Anaerobic fermentation is described as a three-step process according to the biokinetic model, which includes a) extracellular hydrolysis of complex compounds into soluble assimilable substrates, b) transport of soluble assimilable substrates into cells, and c) utilization of assimilable substrates for the cell growth and product formation [3,22–25].

The complex organic compounds are hydrolyzed by extracellular enzymes (hydrolases) excreted by hydrolytic microorganisms [26]. A linear trend is used in the hydrolysis process to account for changes in concentration over time in the hydrolysable substrate such that

$$\frac{dS_h}{dt} = K_i(S_i - S_h) \tag{9}$$

The total of the intracellular and extracellular hydrolysis rate coefficients, K_h, in the conceptual model for the anaerobic digestion assuming there is no diffusional constraint for the transfer of solubilized material out of the damaged cell [2].

Before it can be processed by microorganisms, complex organic material must be reduced to a solution that can be transferred across cell membranes [27]. 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

$$\frac{-dS_h}{dt} = K\big(S_h - S_g\big)X = kS_hX \tag{10}$$

Eqs. (9) and (10) can be rearranged to determine the concentration of hydrolyzed substrate (S_h) by following the equation:

$$S_h = \frac{K_hS_i}{kX + K_h} \tag{11}$$

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

$$X = y(S_{0-i} - S_i) + X_0 \tag{12}$$

The Monod [10] and Michaelis-Menten [28] biokinetic models of bacterial growth were based on the relationship between a specific growth rate and a limited substrate concentration. To resolve the existing limitation in the Monod model, several useful unstructured biokinetic models had evolved for the projection of cell growth in biological wastewater treatment processes. These unstructured growth rate models are dependent on a) the concentration of the substrate (Monod, Tessier, Ming, Sokol-Howell), b) the concentration of the cell and/or the substrate (Contois), and c) the concentration of the substrate and product inhibition (Aiba, Hinshelwood, Ghose-Tyagi, Severly) [11,15]. The model equations have been interpreted empirically to demonstrate their relevance in modeling anaerobic fermentation biokinetics.

The pH value has a significant effect on the degradation process [11], as the presence of ammonia, acclimation, sulfate, and volatile fatty acids (VFAs) results in acidity or alkalinity and hence defines the kind of microbes that live within the anaerobic reactor. The pH inhibition factor (I) is stated as follows [1,7],

$$I = \frac{1 + 2 \cdot 10^{0.5(pH_{min} - pH_{max})}}{1 + 10^{(pH - pH_{max})} + 10^{(pH_{min} - pH)}} \tag{13}$$

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 [29].

Temperature is a critical element for microbial growth in anaerobic fermentation. The temperature has an effect on microbial activities by altering the nutritional needs, the type of metabolism, the content of the biomass, and the rate of reaction [30]. Due to the uncertainty and inapplicability of the Arrhenius equation for the particular parameters, the cardinal temperature model (CTM) might be presented to represent the temperature effect on the anaerobic process [11,31]. The CTM accounts for the experimentally observed point of inflection in the suboptimal range of temperatures [31]. The CTM temperature factor (θ) is stated as follows,

$$\theta = \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]} \tag{14}$$

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. (11), (12), (13), and (14), Eqs. (1-10) can be arranged into Eqs. (15-23) 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, $S_{i\text{-}0}$.

Statistical analysis revealed that the chosen biokinetic models were capable of accurately predicting reactor behavior [12]. Monod's and other biokinetic models' mathematical expressions (Table 1 and Table 2) may 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 may be designed without considering the influence of pH, temperature, or ammonia. Both model configurations were compared in order to anticipate the process's optimal performance since the complexity of microbial activity is

cited as a primary cause for a lack of fundamental information about anaerobic fermentation systems [32].

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

Biokinetic model (Eqs.)	Microbial growth rate on the hydrolyzed substrate	
Monod	$\frac{\mu_{max}}{\mu} = \left[\frac{(K_S Y(S_{0-i} - S_i) + K_S X_0)(kY(S_{0-i} - S_i) + kX_0 + K_h)}{K_h S_i} + 1\right]\left[\frac{1}{f}\right]$	(15)
Tessier	$ln\left[1 - \frac{\mu}{\mu_{max}(f)}\right] = -\left[\frac{K_h S_i}{kK_S[Y(S_{0-i} - S_i) + X_0] + K_h K_S}\right]$	(16)
Sokol-Howell	$\frac{\mu_{max}}{\mu} = \left[\frac{K_S[kY(S_{0-i} - S_i) + kX_0 + K_h]}{K_h S_i} + \frac{K_h S_i}{kY(S_{0-i} - S_i) + kX_0 + K_h}\right]\left[\frac{1}{f}\right]$	(17)
Contois	$\frac{\mu_{max}}{\mu} = \left[\frac{(K_S Y(S_{0-i} - S_i) + K_S X_0)(kY(S_{0-i} - S_i) + kX_0 + K_h)}{K_h S_i} + 1\right]\left[\frac{1}{f}\right]$	(18)
Ming	$\frac{\mu_{max}}{\mu} = \left[\frac{K_S[kY(S_{0-i} - S_i) + kX_0 + K_h]^2}{(K_h S_i)^2} + 1\right]\left[\frac{1}{f}\right]$	(19)
Aiba	$\frac{\mu_{max}}{\mu} = \left[\frac{K_S[kY(S_{0-i} - S_i) + kX_0 + K_h]}{K_h S} + 1\right]\left[\frac{1}{e^{-k_1 P}}\right]\left[\frac{1}{f}\right]$	(20)
Hinshelwood	$\frac{\mu_{max}}{\mu} = \left[\frac{K_S[kY(S_{0-i} - S_i) + kX_0 + K_h]}{K_h S_i} + 1\right]\left[\frac{-P_m}{P - P_m}\right]\left[\frac{1}{f}\right]$	(21)
Ghose-Tyagi	$\frac{\mu_{max}}{\mu} = \left[\frac{K_S[kY(S_{0-i} - S_i) + kX_0 + K_h]}{K_h S_i} + 1 + \frac{K_h S_i}{K_i[kY(S_{0-i} - S_i) + kX_0 + K_h]}\right]\left[\frac{-P_m}{P - P_m}\right]\left[\frac{1}{f}\right]$	(22)
Severly	$\frac{\mu_{max}}{\mu} = \left[\frac{K_S[kY(S_{0-i} - S_i) + kX_0 + K_h]}{K_h S_i} + 1\right]\left[\frac{K_i + P}{K_i}\right]\left[\frac{-P_m}{P - P_m}\right]\left[\frac{1}{f}\right]$	(23)

f is the inhibition factor as follows: the influence of pH (f = I), the influence of temperature (f = θ), the influence of pH-ammonia (f = I), the influence of pH-temperature (f = Iθ), the influence of temperature-ammonia (f = θ), the influence pH-temperature-ammonia (f = Iθ), the influence of ammonia and no influence of pH-temperature-ammonia (non-f or f is negligible). See Table 2 for selected influence effects.

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

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.

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