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Comparative Analyses of Select Microbial Growth Inhibited Rate Models

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Abstract

Food waste is a complex substrate comprising of different mixtures of chemical compounds. The growth rate of micro-organism in such environment will experience inhibition. Consequently, the Monod's model will not be able to describe the growth rate of its bacteria. The Aiba's, Andrews and the Haldane's growth rate models that are accounts for growth rate of bacteria with inhibition were compared. To do this, seven different batch experimentations; each with different initial substrate concentration were carried out at constant temperature. The data obtained during the time course of rection were used to curve fit these model equations using Python software. The results showed that the Aiba's model had the best fit followed very closely by the Andrew and Haldane's models which were essentially the same as indicated by the value of their coefficient of correlation. This is an indication that any of these models are suitable for modelling the growth rate of bacteria in a batch reactor using food waste as the substrate. Notwithstanding, due to the formation multiple steady state formation by the Andrew and Haldane's models, they cannot be used to model a continuous stirred tank reactor. On the other hand, the steady state concentration of the Aiba's model requires a numerical software for ease of solution.

Keywords: Aiba's model, andrew's model, haldane, complex substrate, growth rate models.

INTRODUCTION

Two major problems faced during the modelling of anaerobic reactors is the problem of the specific growth rate model to use and the other being the parameters of the chosen model. The specific growth rate constant is synonymous to the rate constant in normal chemical reaction. However, unlike in normal chemical reaction, in anaerobic digestion, AD, the specific growth rate does not relate to temperature exponentially and hence, cannot be determined using the Arrhenius equation [1].

The Monod's model is an example of non-inhibitory growth model similar to that of Michaelis-Menton's equation and was derived in 1948. Although it is the most widely used due to its simplicity, the Monod's model is faced with several criticism with respect to the growth of microbes in complex or toxic substrates such as municipal solid wastes, industrial effluents and food wastes. The Monod's model only describes the dependence of the rate of biodegradation on biomass concentration [2]. However, when substrates exhibit self-inhibition, the Monod's model fails. Substrate or nutrient inhibition happens when the substrate's concentration exceeds the optimal parameters and decreases the growth rate of the cells. This is quite different from

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substrate limitation that is applicable to the Monod's model. Several growth models have been published to describe substrate inhibition [3–5].

The accuracy of the Monod's kinetics when used for homogenous cell cultures and simple substrates is very high. This accuracy drops when applied to heterogeneous cultures or complex substrates such as that witnessed in biodigestion [6–7]. The Monod model cannot be used to describe the anaerobic degradation of complex substrates such as municipal solid wastes. This is because, the Monod's kinetics was originally developed as a model for the growth of pure bacterial specie in limited nutrient environment and as such does not encounter inhibition as pointed out earlier [8]. For these reasons, he recommended that the Monod's model needs to be modified depending on the specificity and requirements of the process.

Consequently, several modifications as well as other kinetic growth models have been proposed to account for the growth inhibition of microbes as presented in Table 1. These models are classified as structured and unstructured kinetic models.

In the structured kinetic growth model, the genetics, morphology or biochemical attributes that collectively determine the physiological state of the microbes are incorporated. While they have been confirmed to be able to describe the specific growth rate, they are said to be complicated and requires many equations to solve [9].

Unstructured kinetic growth models consider microbes as independent organisms interacting with their environment. They describe bacterial growth rate based on substrate and cell concentration. Table 1 shows some of these models.

Research had described that the specific growth rate μ_o, represents the dynamic behaviour of micro-organisms and it is the most important parameter to control during fermentation. Notwithstanding, microbes cannot adapt to their environment when cultivated in batches.

The half saturation constant K_s is defined as the substrate concentration that produces a rate of $\frac{1}{2}\mu_{max}$. It is very useful in comparing the ability of species to compete for nutrient. At high substrate concentration, it has been reported that cells with higher K_s value will grow faster than those with low K_s and consequently, will dominate the microbial mixed population. The reverse is experienced in low substrate environment as cells with low K_s value will dominate [10]. K_s values can also be used to determine the steady state concentration of unused growth limiting substrate in continuous process.

The inhibition constant K_I, is equal to the maximum substrate concentration required to reduce the maximum rate μ_{max} by half [11]. It is a measure of how potent an inhibitor is.

Table 1. Some Growth Rate Models with Inhibition

Models	**Equation**	**Description**
Models	***For Growth With***	***Note***
Haldane	$\mu_o = \mu_{max}\dfrac{S}{K_s + S + \frac{S^2}{K_{I,S}}}$	
Andrews	$\mu_o = \mu_{max}\dfrac{1}{\left(1+\frac{K_s}{S}\right)+\left(1+\frac{S}{K_{I,S}}\right)}$	
Aiba	$\mu_o = \mu_{max}\left(\dfrac{S}{K_s+S}\right)\left(e^{-\left(\frac{S}{K_{IS}}\right)}\right)$	

Among the several substrate inhibition models available in literature, revealed that the most widely used substrate inhibition model is the Haldane's model as stated in Table 1. This model, was proposed in 1968 as a deviation from the Monod's and incorporates the effect of substrate inhibition on the specific growth rate. Unlike the Monod's model which can be transformed to a linear model, the Haldane's growth model cannot be expressed in linear form. Since the Haldane's equation is nonlinear, although it can be expressed as a polynomial that permits the inclusion of three constants, its parameters were determined by curve fitting.

A batch biodigester assuming the Haldane's equation. However, most of the kinetics parameters were taken from different authors who worked on different biomaterials at different times.

A comparative analysis of different kinetics models using different substrates such as Swine wastewater, Palm oil mill effluent and pharmaceutical wastewater. Their studies reveal that the Tessier's type 1, Moser's and the Moser and Bergter's models had the best fits.

A CSTR and BR respectively for the treatment of municipal solid waste for biogas production, on the assumption that the reaction followed the Monod's growth model. This has been the case of many modeling of anaerobic digestion. However, in its original form, the Monod growth model was developed for a single culture that is growing on single limiting substrate.

No matter the growth model used, one of the key issues involved, is how to determine the parameters of these growth models for design purposes. The parameters of these models are determined using regression analysis.

MATERIALS AND METHODS

Materials

The following materials were used during this research work: nylon bags, blender (shredder/crusher), storage container for cattle rumen's content, digital weighing balance M411L by M-METLAR, refrigerator and food wastes (substrate), which was a blend of several kitchen wastes such as: rice, beans, bread, carrot, cucumber, banana, plantain, baked cassava flakes, meat and fish fragments, spaghetti, etc.

Methods of Determination of Growth Coefficients

The growth model predicts the growth pattern of microbial cells with respect to substrate concentration. Food wastes and cattle rumen content were collected from the fridge and mixed in the ratio $3:1$ to mixed substrate. This inoculation was necessary for all seven (7) different samples since food waste requires some percentage of inoculation for effective biogas production. This mixed substrate was mixed with water to form a batch run (i.e., sample 1) of known concentration.

Six (6) other samples of different concentration were prepared by following the same procedure. These seven (7) samples represented the seven different batches with different initial concentrations viz: $6.24\,gVS/l$, $12.18\,gVS/l$, $33.35\,gVS/l$, $42.62\,gVS/l$, $51.14\,gVS/l$, $63.93\,gVS/l$ and $75.21\,gVS/l$. Each of these were subjected to anaerobic digestion during which the cell and substrate concentrations were regularly recorded.

The relationship between the overall specific growth rate and the cell concentration is defined as:

$$\frac{dX}{dt} = \mu_o X \tag{1}$$

Integrating from the start of the quasi-linear phase (or sometimes exponential phase) to the end of the exponential phase:

$$ln\left(\frac{X}{X_0}\right) = \mu_o(t - t_0) \tag{2}$$

The initial condition (t_0, X_0) used here does not refer to the start of the experiment but the beginning of the quasi-linear phase. As always, t_0 is always zero; since it is the starting time, be it the start of the reaction or the start of the log phase. Similarly, X_0 corresponds to the cell concentration at the start of the quasi-linear phase. Therefore, Equation (2) transforms to:

$$ln\left(\frac{X}{X_0}\right) = \mu_o t \tag{3}$$

For each run, prior to the determination of μ_o, the graph of cell concentration X against time t was first plotted in order to have a pictorial view and identify where quasi-linear phase. This is because, in its original form, the specific growth rate is defined for the log phase and not the entire phase. Hence, the analytical use of Equation (3) will be misleading without knowledge of the quasi-linear phase as revealed in the concentration-time graph.

Finally, the parameters of different growth models are determined by curve fitting different growth models using any numerical software that can handle iterative nonlinear least squares such as Python.

Steady State Implication of Haldane's Model

For reactions that follow the Haldane's growth kinetics, at steady state, $\mu_o = D$ and hence, the equation becomes:

$$D = \frac{\mu_{max} S^{\square}}{K_s + S^{\square} + \frac{S^{\square 2}}{K_I}} \tag{4}$$

Expressing Equation (4) as a quadratic in the steady state concentration, $S^{\square}$:

$$S^{\square 2} + \left(K_I - \frac{\mu_{max} K_I}{D}\right) S^{\square} + K_s K_I = 0 \tag{5}$$

The steady state substrate concentration $S^{\square}$, obeying the Haldane's growth model can be shown to be is defined by Equation (6).

$$S^{\square} = \frac{1}{2}\left(-\left(K_I - \frac{\mu_{max} K_I}{D}\right) \pm \sqrt{\left(K_I - \frac{\mu_{max} K_I}{D}\right)^2 - 4K_s K_I}\right) \tag{6}$$

Equation (6) shows that the Haldane's growth model exhibits multiple steady states. This implies that if a reaction follows the Haldane's model, it will be difficult to estimate its steady state concentration when carrying out optimization analysis as the output from the numerical software at any time might not be a true representation of the process. Similar solution and behaviour are observed when the Andrew's model is solved for its steady state concentration.

RESULTS AND DISCUSSION

Figure 1 to Figure 7 shows the value $(y = \mu)$ of the specific growth yield ran for each initial substrate concentration. The results show that specific growth rate was low initially which can be attributed to deficiency in the amount of substrate available. Thereafter, it increased following increase in the amount of substrate. Further experimentation with yet higher initial substrate concentration led to decline in the specific growth rate. Several authors have also observed this trend for different substrates. A plot of the values of these specific growth rates against their respective initial substrate concentration (Figure 8) shows a departure from the Monod curve. This confirms that the Monod's model is not effective in describing the growth of heterogeneous microbes in complex substrate.

To determine the particular growth model the bacteria followed most, the data of Figure 8 were used to fit different growth models and results summarized in Table 2.

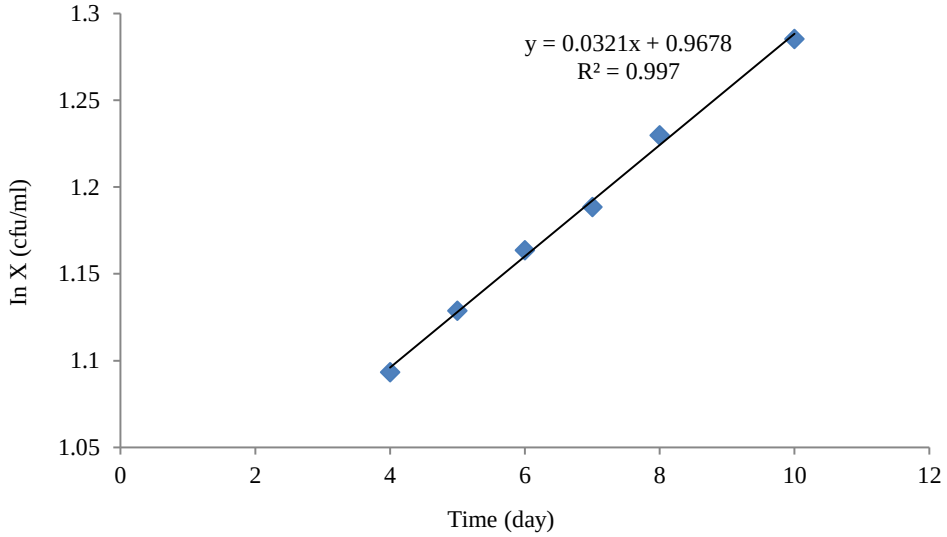

Figure 1. Determination of Specific Growth Rate for Batch Run 1.

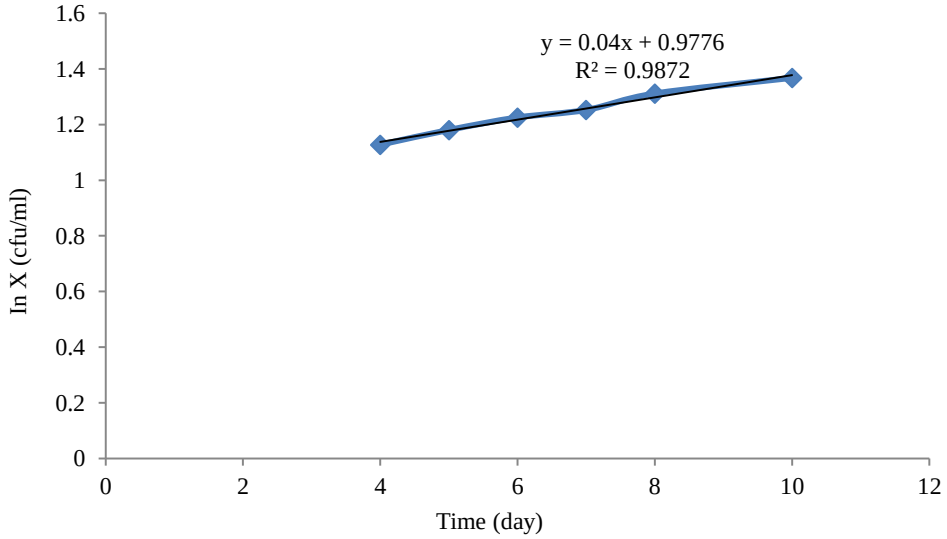

Figure 2. Determination of Specific Growth Rate for Batch Run 2.

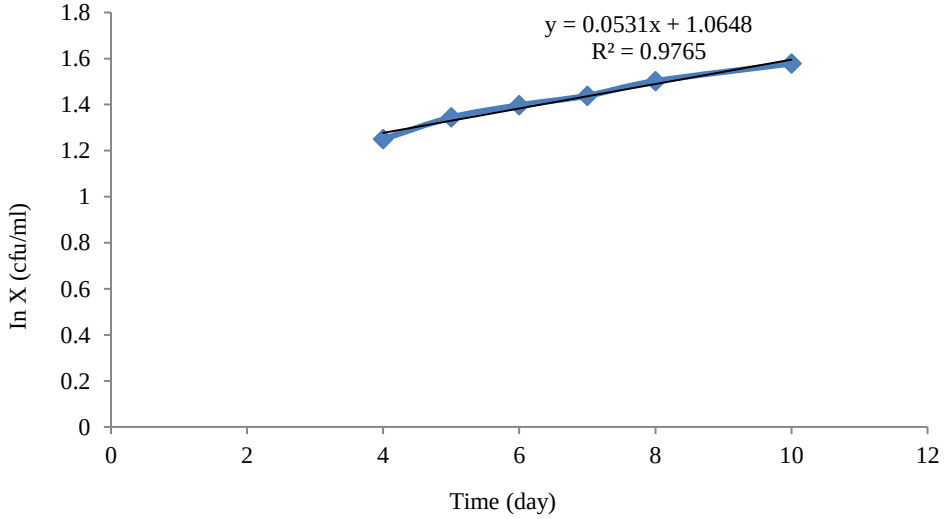

Figure 3. Determination of Specific Growth Rate for Batch Run 3.

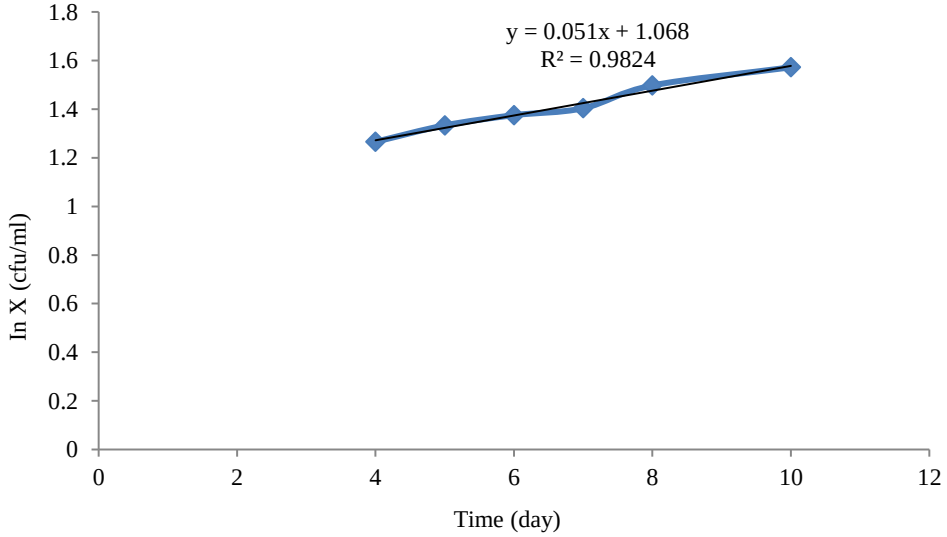

Figure 4. Determination of Specific Growth Rate for Batch Run 4.

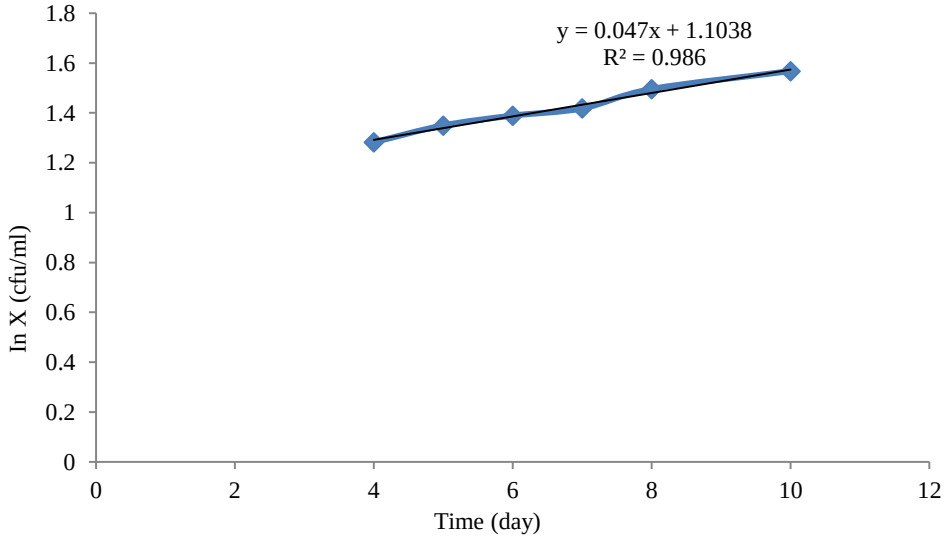

Figure 5. Determination of Specific Growth Rate for Batch Run 5.

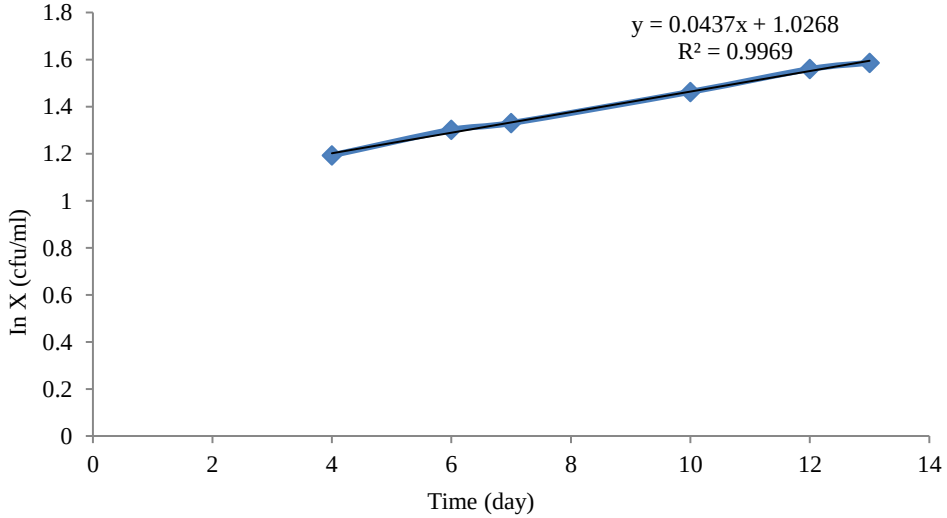

Figure 6. Determination of specific growth rate for batch Run 6.

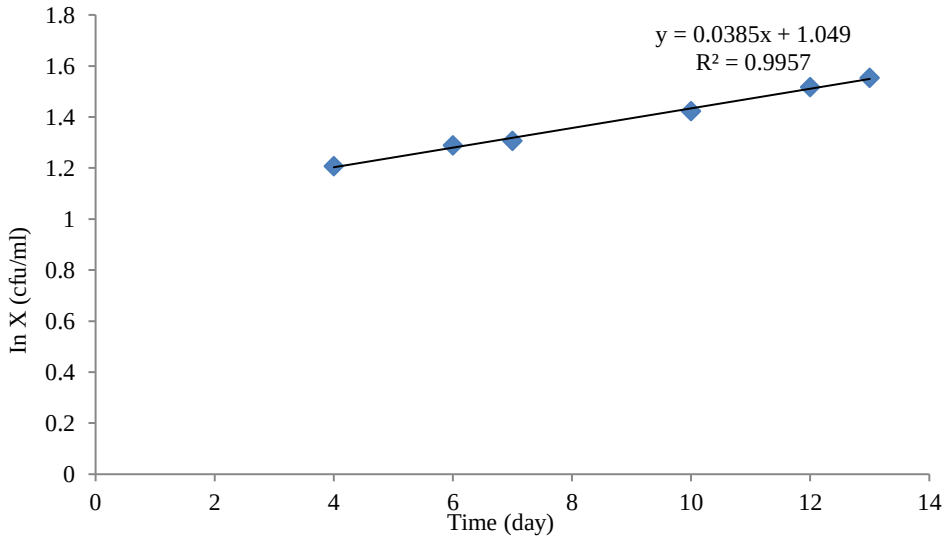

Figure 7. Determination of specific growth rate for batch Run 7.

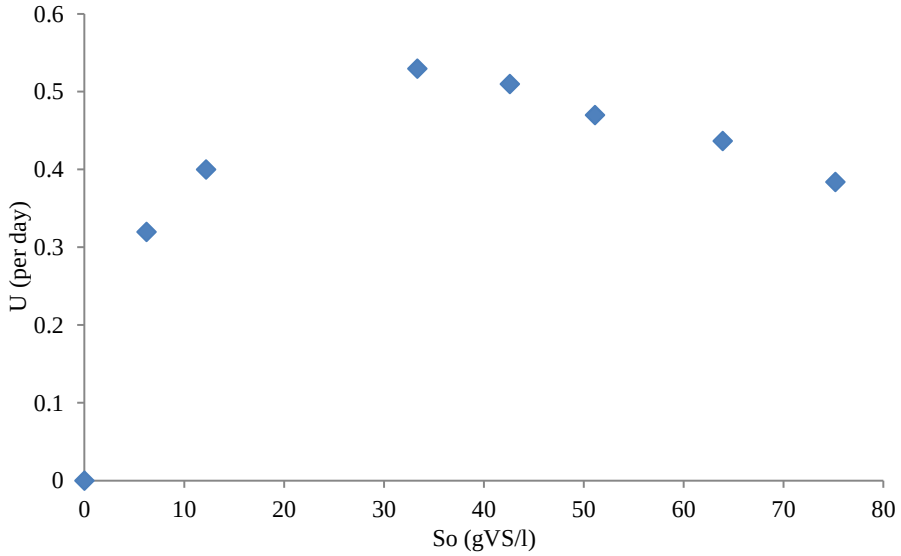

Figure 8. Specific Growth Rate Curve of the Microbes.

Table 2. Results of Different Growth Models kinetics.

Model	**Equation**	$\mu_{max}(d^{-1})$	$K_s(gvs/l)$	n	R^2
Models	***For growth with***	μ_{max}	K_s	$K_{I,S}$	R^2
Haldane	$\mu_o = \mu_{max}\frac{S}{K_s + S + \frac{S^2}{K_{I,S}}}$	0.1246	19.2649	40.5462	0.9323
Andrews	$\mu_o = \mu_{max}\frac{1}{\left(1+\frac{K_s}{S}\right)+\left(1+\frac{S}{K_{I,S}}\right)}$	0.2491	38.5296	20.2732	0.9323
Aiba	$\mu_o = \mu_{max}\left(\frac{S}{K_s+S}\right)\left(e^{-\left(\frac{S}{K_{IS}}\right)}\right)$	0.1223	15.6493	86.7868	0.9568

Table 3 is a summary of the outcome of the different growth models. The Contois model was found to have the poorest fit with an R^2 value of 0.4408. This shows that the Contois growth model cannot be used to describe the growth of microbes in toxic substrate or high substrate concentration with complex nutrient composition such as food wastes, animal dung as well as their combinations.

The Aiba's model, sometimes referred to as the Aiba-Edward model had a satisfactory fit with an R^2 value of 0.9568. This confirms its ability to describe cell growth with inhibition as well as the lag and death phases of growth. While agreeing that the Aiba's equation gave satisfactory results stated that the model deviates slightly at certain critical value of toxins or substrate concentration. The veracity of such report is worthy of further investigation. The challenge with the Aiba's equation is with finding an expression for the steady state substrate concentration analytically. Worthy of note is that when $S^{\square} \ll K_I$, as in this work, the Aiba's equation approximates to Monod's. Therefore, while the Monod model cannot be used to describe a batch process, it can be used to describe a steady state process.

The Haldane's growth model showed a very good fit as the Andrews with an R^2 value of 0.9323. The advantage of this model is that it has the ability to describe all growth phases as well as able to describe growth rate at both high and low substrate concentration the Haldane's model has hardly failed in any experimental data. The only problem faced with the Haldane's growth equation is that it exhibits multiple steady state concentration which makes it difficult to use in modelling a CSTR or chemostat.

The Andrew's model had the same result with Haldane's equation up to four (4) decimal places with R^2 value of 0.9323. This should not come as a surprise as both model equations are very similar. Just like the Haldane's growth model, the Andrew's model is able to describe substrate inhibition as well as all the growth phases of bacterial. In fact, the models are very much similar as it also exhibits multiple steady state concentration. Hence, as always, there was high accuracy and agreement between their R^2 values.

The K_s have been somehow infrequently determined, and most reported values lack information on their accuracy. Contrariwise, the method of cell count adopted here, tries to mitigate the error involved in measuring cell concentration in solid suspended media. Furthermore, the Code of Federal Regulations on Protection of Environment, opined that when the growth model parameters such as K_s and K_I are negative, then these parameters may not be used and such experimental procedure cannot be used to evaluate the performance of a biological treatment. In line with this, the results of these parameters justify the procedures used in this work. Notwithstanding, the results obtained here are not sacrosanct. But will depend upon several conditions such as the feedstock, the concentration (water to substrate ratio), temperature, pH of the system, agitation, etc.

CONCLUSION

Comparative analysis of the Monod's and Haldane's growth rate model was investigated.

Several growth models have been proposed to predict the growth kinetics of microbes both in substrate limited as well as in toxic and complex substrate digestions. It was found that the Monod's model was one of the least accurate in predicting cell growth in food waste digestion. Although the Aiba's model was found to be the best, any of Andrew or Haldane is a good representation of microbial growth in food waste. Notwithstanding, the complex nature of these three model equations, makes it difficult to use any of them in modelling a CSTR. Andrew's and Haldane's exhibit multiple steady states while computer software is required to provide a numerical solution of the Aiba's model. Hence, any of the other growth models such as Tessier, Moser or the Monod's may be used as an alternative during preliminary study of a CSTR.

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