Predicting Microbial Growth in Anaerobic Digester Using Gompertz and Logistic Models

SYLVIA INJETE MURUNGA[1], FESTUS WERE[2]
[1]*Department of Agricultural and Biosystems Engineering, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*
[2]*Department of statistics and actuarial sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

Abstract- ***Models plays a vital role in understanding microbial growth in wastewater treatment and bioremediation processes, as is, in safe food production, microbe-mediated and mining among others. However, it is also gaining popularity in optimization designs. A study was undertaken to predict microbial growth in anaerobic digestor using Gompertz and logistic models. The objective was to determine the growth parameters and compare the performance of these primary models in anaerobic digestion (AD). Three isolates from brewery waste water closely related to Bacillus subtilis, Bacillus methylotrophicus and Lysinibacillus species were used as inoculum and their growth monitored based on optical density (OD) at the same conditions but different initial cell concentration. Microbial population growth data were fitted to the modified logistic function and Gompertz function using Marquardt algorithm and the comparison was based on both the Alkaike Information Criterion value (AIC), Residual Sum of Squares and*** R^2***values.***

All the models had a high goodness of fit (R^2***> 0.93) for all growth curves for three isolates, in all the cases. However, Gompertz model was accepted in 66.67% of the cases based on the AIC values and also supported by the*** R^2 ***> 0.95values and small RSS values. The models provided knowledge to define the growth of the methanogenic community in a bio-digester as a function of time, which could be used for maximum utilization of the exponential phase of the microbial growth for production of biogas. This indicates the practicality of applying Gompertz model to actual anaerobic digestion of brewery waste water. Growth parameters like the rate of increase in the number of cells per unit time and lag time were determined from the models.***

Indexed Terms- ***Anaerobic digestion, Biogas, Gompertz, Logistic, Microbial growth Models.***

I. INTRODUCTION

As the utmost common process for the biological treatment of wastewater and biogas production, AD has gained significant importance, albeit problems such as low methane yield and process instability, preventing this technique from being widely used [1], [2]. This process requires synergistic efforts of various microorganisms in various steps including hydrolysis, acidogenesis, acetogeneisis and methanogenesis, where the products of one step are utilized in the next one finally culminating in the production of biogas [3]

Acid forming and the methane forming microorganisms vary broadly in terms of structure, nutritional needs, growth kinetics, and sensitivity to environmental conditions. Thus, balance between these two groups of microorganisms has to be maintained ,to reduce possibilities of reactor instability [1]. In addition, the low growth rates and the susceptibility of the organisms to toxins enhances the difficulties in the optimization of methanogenesis [4]. Investigation of these methanogens can not only assist in the classification but also in the optimization of the AD systems [5].Thus, the study builds on the primary microbial growth models developed by Gomperzt and Logistic to describe the growth of the methanogenic community in a bio-digester as a function of time.

Microbial models can be classified as primary, secondary or tertiary [6], [7]. Primary models

describe how the number of microorganisms in a population changes with time under specific conditions. Secondary models relate the primary model parameters to environmental or intrinsic variables such as temperature or pH. Tertiary models combine primary and secondary models with a computer interface providing a complete prediction tool [8], [9]. Several primary growth models exist in the literature, such as the models by Gompertz, Richards, Stannard et al., Schnute, and the logistic model among others [10], [11].These mathematical expressions have been modified to which give biological meaning to the parameters as illustrated in Table 1,and they differ in "ease of use" and number of parameters in the equation [12].

Model selection seems to be biased though, Gompertz, Richards, and logistic, are the most commonly used [9], [13]. The models have different number of growth parameters defined by the growth curve thus there may be difference in the results obtained using different growth models [11]. The behaviour of different growth models have been compared in literature ranging from different mathematical measures of goodness of fit and/or other statistical criteria [14]. Direct comparisons of specific growth parameters as predicted by various models have also been explored with different conclusions, hence, there is substantial disparity in literature on which is the best-fitting model for predicting microbial growth [13], [15].The modified stannard equation appears to be the same as the modified Richards equation (Table 1), with four growth parameters (A, μ_{max}, λ and υ).The υ ,in the four parameter models represents the shape parameter which is difficult to explain biologically, and are significantly better to use when a large number of datum points are collected. However, three parameter models have more degrees of freedom for the parameter estimates which can very useful when applied to growth curves of small number of measured points [11].

Plotting the population growth verses time data yields a typical bacterial growth curve which is usually divided into the lag phase, exponential phase, stationary phase and the death phase as illustrated in Figure 2 [16].

- Lag Phase.

After inoculation of the cells into fresh medium, the population remains temporarily unchanged during this phase. The cells may grow in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increase in metabolic activity, though no apparent cell division occurs. The duration of the lag phase depends on number of factors, including, but not limited to the initial cell concentration, the time required to recover in the transmission from physical damage or shock, time required for synthesis of essential coenzymes or division factors, and time required for synthesis of new enzymes that are necessary to metabolize the substrates present in the medium. In this phase, the growth is approximately equal to zero, thus;

$$dN/dt = 0 \quad [1]$$

Where N represents the number of cells.

- Exponential (log) phase.

The cells divide at a constant rate by binary fission and grow by geometric progression depending on the growth medium and the conditions of incubation [17]. The rate of exponential growth of a bacterial culture is expressed as generation time (doubling time**)** of the bacterial population. The growth can be represented as;

$$Z^0 \rightarrow Z^1N \rightarrow Z^2N \rightarrow Z^3N \rightarrow Z^4N \rightarrow Z^5N \rightarrow Z^nN$$

Where n represents the number of doublings occurred after some time interval.

Thus,

$$n = t/t_d \quad [2]$$

Where, t_d is the doubling time in hours. It follows therefore that the number of cells present at time t, in relation to the initial population is given as;

$$N_t = N_0 2^n \quad [3]$$

Where, n= represents the number of generation,

N_t =Final number of cells

N_o =Initial number of cells

Substituting the value of n in equation [3] gives;

$$N_t = N_0 2^n = N_0 2^{t/t_d} \quad [4]$$

Similarly,

$$N_t / N_0 = 2^{t/t_d} \quad [5]$$

Taking logarithms,

$$In(N_t / N_0) = 2^{t/t_d}$$

Which is the same as,

$$\frac{(InN_t - InN_0)}{t} = \frac{0.693}{t_d} \quad [6]$$

Plotting the natural logarithm of the number of cells against time of incubation should yield as straight line whose slope is equivalent to $\frac{0.693}{t_d}$ Thus,

$$d(InN)dt = 0.683 / t_d \quad [7]$$

The specific growth rate constant (the rate of increase in the number of cells per unit time) can be given by;

$$\frac{d(InN)}{dN} \times \frac{dN}{dt}$$

$$\frac{d(InN)}{dN} \times \frac{dN}{dt} = \frac{1}{N} \times \frac{dN}{dt} = \frac{0.683}{t_d} \quad [8]$$

Where;

$\frac{1}{N} \times \frac{dN}{dt}$ is the specific growth rate, μ and the units are in reciprocal hours (h^{-1}).

- Stationary phase.

The growth of bacterial usually goes through a phase in which the specific growth rate starts at a value of zero and then accelerates to a maximal value (μ_{max}), in a certain period of time. The asymptote is the maximal log10 N value reached resulting to maximum population. In a batch culture, population growth is controlled by a number of factors without limitation, to exhaustion of available nutrients; accumulation of inhibitory metabolites or end products; and exhaustion of space thus limiting the exponential growth [16], [18]. During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of dormancy.

- Death phase.

During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase, and cannot be observed if counting is done by turbidimetric measurements or microscopic counts.

Generally, data on growth curves are necessary to define and construct predictive models in anaerobic digestion. For reduction of measured data to important growth parameters in microbial growth, models play an important role as opposed to using linear regression [6], [9], [13], [19].

II. MATERIALS AND METHODS

- Microorganism and culture medium

Three isolates closely related to Bacillus subtilis, Bacillus methylotrophicus and Lysinibacillus species as identified through sequencing from brewery wastewater were used as inoculum [20], [21]. Freshly cultured 12 hour old cells of were used in this experiment. 200µl of the cells were inoculated in 800µl brewer Thyglycollate media after autoclaving at 121ºC and incubated in a Labtechdhaihan shaking incubator at 250 rpm. The initial turbidity was observed by taking the $OD_{600\ nm}$ values using a spectrophotometer at incubation time of zero for all the organisms. Incubation was done at 37ºC and the OD values were taken at the time intervals of 0, 1, 2, 4, 6, 15, 18, 22, 24, and 28 hours respectively in triplicates until the readings were constant. To reduce effect of cell multiplication during turbidity determination, the samples were placed in a freezer until the readings were recorded.

- Fitting of the experimental data

Non-linear regression in R programming language and Marquardt algorithm were used to fit the microbial population growth data to the modified logistic function and Gompertz function as illustrated in equation [9] and [10] respectively.

$$\log 10N(t) = \log 10N_0 + \frac{A}{(1 + \exp[\frac{4\mu_{max}}{A}(\lambda - t) + 2])} \quad [9]$$

$$\log 10N(t) = \log 10No + A\exp\{-\exp[\frac{\mu \max .e}{A}(\lambda - t) + 1]\} \quad [10]$$

Where A is given by equation [11]

$$A = \log 10 \frac{N \max}{No} \qquad [11]$$

μ_{max} is as illustrated in equation [8], while λ and t represent the lag time and time of incubation respectively. Marquardt algorithm minimizes the sum of the squares of the differences between the predicted and measured values. It automatically calculates starting values by searching for the steepest gradient of the curve as illustrated in equation [8] between four datum points (estimation of μmax), by intersecting this line with the x axis (estimation of λ), and by taking the final datum point as estimation for the asymptote (A)[11], as illustrated in equation [11]. The algorithm then calculates the set of parameters with the lowest residual sum of squares (RSS) and their 95% confidence intervals. The death phase was not considered in this study.

- Model comparison

Data fits obtained by using the growth models were compared statistically by the use of Akaike Information Criterion (AIC) [22] based on information theory, r^2,and RSS [9], [10],with 95% confidence limits. The AIC is defined by equation [12].

$$AIC = N \ln \frac{SS}{N} + 2K \qquad [12]$$

Where N represents the number of data points, K is the number of parameters fit by the regression plus one, since regression is estimating the sum of squares as well as the values of the parameters, and SS is the sum of square of the vertical distances of the points from the curve. An AIC value can be positive or negative and the sign doesn't have a meaning since it can be changed using different units to express data. Models were compared by evaluating the difference between the AIC values in which the model with the smallest AIC values was taken as the most likely to be correct.

III. RESULTS AND DISCUSSION

Table 2 shows the growth parameters as estimated by the Gompertz and logistic models for the growth curves of the three isolates closely related to Bacillus subtilis, Bacillus methylotrophicus and Lysinibacillus species plotted with the log of N values. All the models gave a good fit of data, and provided the values that could be expected for growth parameters of the three growth curves selected of this microorganism, although they gave different estimates of the growth parameters.

Figure 1 shows the growth curve fitted with both Gompertz and logistic models for isolate closely related to Bacillus Subtilis. When comparing the growth rate values given by the two models Table 2, Gompertz gave the lowest growth rate of (0.355-2.680) cells h^{-1} with a lag time of (3.762-4.725) hours while logistic had a growth rate of (0.397-1.890) cells h^{-1} with a lag time of (3.982-5.588) hours. The R^2 value for the logistic model was as low as 0.979, with a high SSE value of 0.026 as compared to values from Gompertz model. The logistic model was also found to have a high AIC value thus, Gompertz model was found to be the most likely model to be correct as it had the best fit for the isolate.

For the isolate closely related to the Lysinibacillus .Sp, Logistics model gave the highest growth rate values of (0.245-2.014) cells h^{-1} with a lag time of (3.183-5.620) hours while Gompertz had a growth rate of (0.257-2.217) cells h^{-1} with a lag time of (3.172-4.809) hours. The R^2 value for the logistic model was as low as 0.944 , with a high RSS value of 0.087 as compared to R^2 and RSS value of 0.962 and 0.0447 respectively for the Gompertz model. Gompertz model however, had a lower AIC value thus, it was found to be the most likely model to be correct for isolate closely related to Lysinibacillus. Sp.

Gompertz model had the lowest values for the estimated growth parameters as (0.117-0.637) cells h^{-1} for growth rate and lag time of (3.648-5.599) hours as compared to (0.167-1.939) cells h^{-1} and (4.252-5.873) hours respectively for logistic model, Figure3.The AIC value and RSS value for the Gompertz model were also observed to be lower. However, the R^2 value for all the models were above 0.995. Basing on the AIC value, the Gompertz model was found to be the most likely to be correct.

All the models provided a high goodness of fit (R^2> 0.93) for all growth curves for three isolates, in all

the cases. The differences between the Gompertz and logistic models were not significant. This is in line with the findings of Longhi et al., 2013. However, Gompertz model was accepted in 66.67% of the cases based on the AIC values and also supported by the R2> 0.95values and small RSS values. The lag stages, slopes and constant growth stages for all the growth curves representing the growth of these microorganism were in line with, lag, exponential and stationary growth phases as reported in literature [6], [9], [11], [13]. The lag time and growth rates were also different although all the microorganism were grown in the same conditions, depicting different adaption times for different microorganisms [18], [23].This information could be useful in the determination of the sludge retention time for the methanogenesis step in order to allow maximum contact of the feedstock and the bacterial mass and to minimize transport problems related to toxins with respect to substrate compounds, intermediate and end products[1], [4].

CONCLUSION

The models provided knowledge to define the growth of the methanogenic community in a bio-digester as a function of time, which could be used for maximum utilization of the exponential phase of the microbial growth for production of biogas. This indicates the practicality of applying Gompertz model to actual anaerobic digestion of brewery waste.

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Table 1: Primary microbial growth models and their biological modified forms

MODEL	MATHEMATICAL EQUATION	MODIFIED EQUATION
Logistic	$y = \frac{a}{[1+\exp(b-cx)]}$	$y = y_o + \frac{A}{\{1+\exp[\frac{4\mu_{\max}}{A}(\lambda - t)+2]\}}$
Gompertz	$y = a - \exp[-\exp(b-cx)]$	$y = y_o + A\exp\{-\exp[\frac{\mu_{\max}.e}{A}(\lambda - t)+1]\}$
Richards	$y = a\{1+\upsilon.\exp[k(\tau - x)\}_{(-\frac{1}{\upsilon})}$	$y = y_o + A\{1+\upsilon.\exp(1+\upsilon).\exp[\frac{\mu_{\max}}{A}.(1+\upsilon)(1+\frac{1}{\upsilon}).(\lambda - t)]\}(-\frac{1}{\upsilon})$
Stannard	$y = a\{1+\exp[-\frac{(1+kx)}{p}]\}_{(-p)}$	$y = y_o + A\{1+\upsilon.\exp(1+\upsilon).\exp[\frac{\mu\max}{A}.(1+\upsilon)(1+\frac{1}{\upsilon}).(\lambda - t)]\}(-\frac{1}{\upsilon})$
Schnute	$y = \{\mathbf{y}_1^b + (y_2^b - y_1^b).\frac{1-\exp[-a(t-\tau_1)]}{1-\exp[-a(\tau_2-\tau_1)]}\}\frac{1}{b}$	$y = y_o + (\mu\max\frac{(1-b)}{a})[\frac{1-b.\exp(a.\lambda+1-b-at)}{1-b}]\frac{1}{b}$

Source: modified from Zwietering, 1990

Legend; a, b, c are mathematical parameters, A is the asymptote of growth curve when population reaches maximum, $\mu\max$ is the maximum of specific growth rate, λ is the lag time.

Table 2: Estimated growth parameters and their 95%confidencelimits for isolates closely related to Bacillus subtulis, Lysinibacillus.spand Bacillus methylotrophicus

Isolate	Growth Model	Initial Concentration (No)	Asymptote A(cells)	Growth rate(μmax)cells h-1	Lag time (λ)(h)	Akaike information criterion (AIC)	R^2	SSE
B.Subtilis	Gompertz	9.204	9.9	0.743	4.702	-26.253	0.986	0.016
		(9.141 - 9.232)	(9.863 - 9.933)	(0.355 - 2.680)	(3.763 - 4.725)			
	Logistic	9.204	9.9	1.548	5.468	-25.252	0.979	0.026
		(9.158 - 9.227)	(9.863 - 9.933)	(0.397 - 1.890)	(3.982 - 5.588)			
Lysinibacillus.sp	Gompertz	9.204	9.916	7.05 x 10-1	4.719	-15.212	0.962	0.047
		(9.144 - 9.252)	(9.880 - 9.980)	(0.257 - 2.217)	(3.171 - 4.809)			
	Logistic	9.204	9.916	1.64	5.523	-15.212	0.944	0.087
		(9.145 - 9.258)	(9.876 - 9.979)	(0.245 - 2.014)	(3.183 - 5.620)			
B.methylotrophicus	Gompertz	8.901	9.733	0.238	4.733	-29.625	0.993	0.011
		(8.865 - 8.930)	(9.704 - 9.759)	(0.117 - 0.637)	(3.648 - 5.599)			
	Logistic	8.903	9.733	0.971	5.693	-29.588	0.981	0.031
		(8.865 - 8.935)	(9.705 - 9.766)	(0.167 - 1.939)	(4.252 - 5.873)			

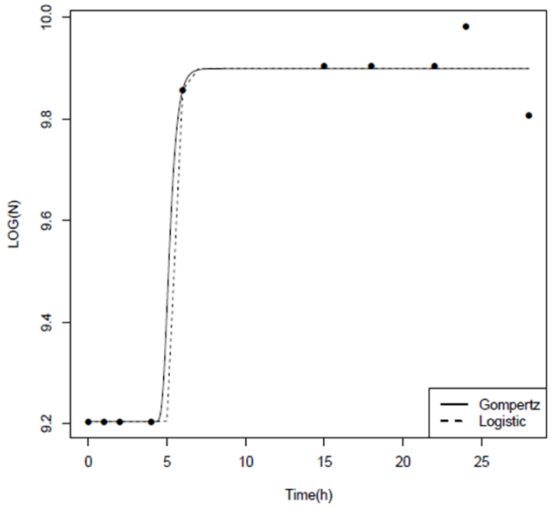

Figure 1: Growth curve for isolate closely related toBacillus subtilis at 37 ° C and pH 7.2 fitted with the Gompertz and logistic model

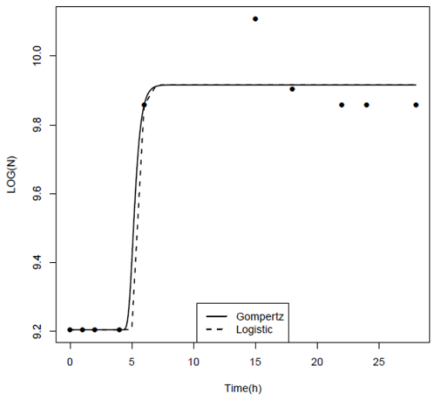

Figure 2Growth curve for isolate closely related to Lysinibacillus. Sp at 37 C and pH 7.2 fitted with the Gompertz and logistic model:

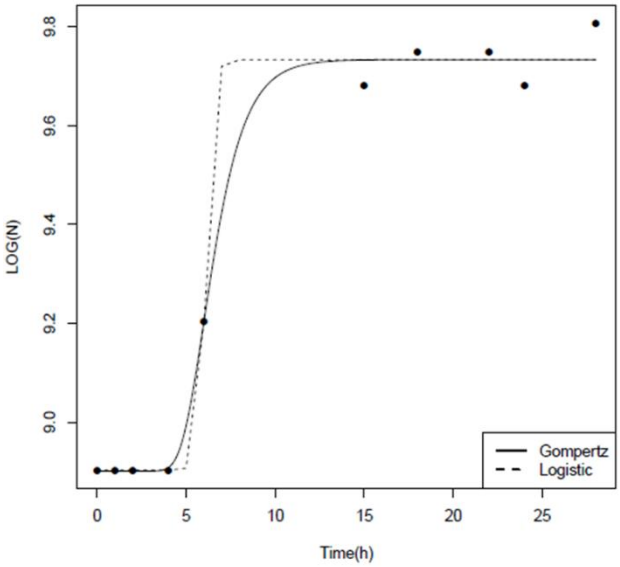

Figure 3: Growth curve for isolate closely related to Bacillus Methylotrophicus at 37 ° C and pH 7.2 fitted with the Gompertz and logistic model

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