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Determination of Growth Parameters of Butyric Acid-Degrading Bacterium, *Achromobacter xylosoxidans,* as a Function of Constant Temperatures in Batch System

John B.J. Njalam'mano*, Evans M.N. Chirwa

University of Pretoria, Department of Chemical Engineering, Water Ultilisation and Environmental Engineering Division, Private Bag X20, Hatfield, Pretoria 0028, South Africa.
chinamvuu@yahoo.co.uk

Achromobacter xylosoxidans is one of the bacterial strains that have the capability to degrade butyric acid which significantly contribute to malodours from pit latrines emissions. There is an increasing interest in being able to predict the consequences of its growth on butyric acid degradation for remediation of malodour emissions from pit latrines. The objective of this work was to elucidate the effect of temperature on butyric acid degradation by *A.xylosoxidans* and to estimate microbiologically relevant parameters at dissimilar isothermal conditions under batch conditions. The experiments were carried out by inoculating 1 mL of bacterial culture into 150 mL each of MSM supplemented with 1,000 mgL^{-1} of butyric acid as a sole carbon source in a sterile 250 mL Erlenmeyer volumetric flask in triplicates. The temperatures were set at 25, 30, 35 and 40 ^oC with initial medium pH of 7 and at agitation rate of 110 rpm. The values of microbiological parameters were obtained by the application of modified Gompertz and modified Logistic sigmoidal models. It was found that the bacterial strain was able to utilise butyric acid as a sole source of carbon at a wide range of temperatures. Both models fitted described most of the experimental data sufficiently to each individual growth curve. The values of the maximum growth rate (μ_{max}) and lag time (λ) obtained using the modified logistic model were higher than the modified Gompertz model. In the cases investigated, the modified logistic model was statistically sufficient to describe the growth data of *A. xylosoxidans* and its application was easy.

1. Introduction

Pit latrines are still the predominant onsite method of hygienic containment, proper treatment and disposal of human excreta in low-income settings, particularly in Africa and India. This is because of their low-cost, simple to construct and serve the purpose of hygienic containment and disposal of excreta (Jenkins et al., 2015). An accumulating body of literature has established that pit latrines act as a primary barrier to faecal-oral disease transmission, whereby pathogens in faecal matter from one host are introduced orally to another host. Pit latrines, however, should be widely adopted and maintained over time and consistently used in order to improve population health. One of the most important impediments, however, with on-site sanitation facilities such as pit latrines is that they generate offensive smells which deter their adoption, investment and consistent use (Rheinländer et al., 2013). This could potentially lead to open defecation despite having pit latrines that cause negative impacts on public health and environment.
Butyric acid ($C_4H_8O_2$) is one of the key important odorants that is generated from pit latrines in significant concentrations (Chappuis et al., 2016). As an individual chemical compound, butyric acid has a distinguishing smell of high odour nuisance values which can be readily detected even at very low concentrations (Ali et al., 2000). Although many odour abatement technologies and strategies exist, biological treatment is still viewed as the most attractive due to its low operation and maintenance costs, reliability and environmental friendliness (Otten et al., 2004). In a recent study, *Achromobacter xylosoxidans* isolated from pit latrine faecal sludge was one of the bacterial strains that had the highest potential for application in biodeodorisation of butyric acid (Njalam'mano and Chirwa, 2018).

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The inherent environmental conditions such as pH, temperature and water activity have capacity to stimulate or retard bacterial growth. Temperature is a factor that can greatly vary within pit latrines (Nabateesa et al., 2017). This can affect the butyric acid deodorisation efficiency of the identified bacterial strains. Since different temperatures affect the bacterial growth dynamics during any bio-process, the use of mathematical model is a useful tool to predict the behaviour of the bacterial strains. Currently, there is no information in literature of the growth behaviour of *A. xylosoxidans* under different isothermal conditions with butyric acid as a sole source of carbon. To better estimate and optimise the biodeodorisation process of butyric acid under different isotherm conditions, growth kinetic modelling is needed to provide crucial information about the microbiological consequences of environmental temperature. Hence, in this work two sigmoidal models which are widely used and discussed in literature are applied to estimate the parameters of microbiological relevance as a function of constant temperatures. Furthermore, for prediction purposes the growth parameters have to be properly estimated hence the performance of models were compared using different statistical indicators to select the best fit model.

2. Materials and methods

2.1 Bacteria

The bacterium identified as *Achromobacter xylosoxidans* isolated from pit latrine faecal sludge in South Africa was used in this study. The bacterial cells were kept in polypropylene tubes at the temperature of -80 °C in mineral salt medium (MSM) prepared according to Roslev et al.(1998) containing 20 % (v/v) glycerol for later use.

2.2 Inoculum preparation

The bacterial cells frozen were partially thawed at 35 ^{o}C in static incubator and pre-grown in 150 mL MSM supplemented with 500 mgL^{-1} butyric acid in 250 mL Erlenmeyer flask. The flask was incubated at 30 ^{o}C, agitated at 110 rpm in the temperature controlled rotary incubator in the dark. The cells were harvested at mid-exponential phase by centrifugation at 9,000 rpm at 4 ^{o}C for 10 min. The centrifugation along with rinsing of the cells in 0.85 % NaCl physiological solution was performed thrice. The final concentration of the harvested cells was adjusted to 2.0(OD_{600}).

2.3 Growth conditions

To evaluate the effect of incubation temperature on the growth variability of the bacterium, 1 mL of the inoculate of optical density (OD) of 2.0 at 600 nm was added to 150 mL MSM supplemented with 1,000 mgL^{-1} butyric acid as a sole source of carbon and energy (with its pH adjusted to neutral) in 250 mL Erlenmeyer flask. Abiotic MSM with the same butyric acid concentration was used as control in triplicates. The growth was assessed at four constant temperature levels of 25, 30, 35 and 40 ^{o}C. 2 mL of the samples were aseptically withdrawn at a predetermined time interval of 4 h until the culture reached its stationary growth phase or death phase. The absorbance was measured at a single wavelength of 600 nm using a UV Lightwave II spectrophotometer (Labotec, South Africa) in a macro quartz cuvette. All the experiments were performed in triplicates. Abiotic MSM with the same butyric acid concentration was used as control in triplicates.

2.4 Primary models

Two re-parameterised modified logistic and Gompertz models with their equations, Eqs(1) and (2), respectively, and their modified equations, Eqs(3) and (4) (Zwietering et al., 1990), respectively, were used in this work.

$$y = \frac{a}{\{1 + exp[-b(t-m)]\}} \tag{1}$$

$$y = a.exp\{-exp[-b(t-m)]\} \tag{2}$$

$$y = \frac{A}{\left\{1 + exp\left[\frac{4\mu_{max}}{A}(\lambda - t) + 2\right]\right\}} \tag{3}$$

$$y = Aexp\left\{-exp\left[\frac{\mu_{max}e}{A}(\lambda - t) + 1\right]\right\} \tag{4}$$

where y is the decimal logarithm of optical density at time, t (h), a is the optical density value of the higher asymptote (dimensionless), m is the time at which the absolute growth rate is maximal (time at inflection) (h), and b is the is the relative maximum growth rate determined at time,m.(h^{-1}) (the slope of tangent to the curve

at m), A is the asymptotic optical density valu as t decreases indefinitely, λ is the lag time and μ_{max} is the maximum specific growth rate. From the above equations parameters of microbiological relevance i.e. Asymptotic value (A) is the same as a while the maximum growth rate (μ_{max}) and lag time (λ) were derived as Eqs. (5) and (6) for the modified logistic model and Eqs(7) and (8) for the modified Gompertz model (Mytilinaios, 2013)

$$\mu_{max} = \frac{bc}{4} \tag{5}$$

$$\lambda = m - \frac{2}{b} \tag{6}$$

$$\mu_{max} = \frac{bc}{e} \tag{7}$$

$$\lambda = m - \frac{1}{b} \tag{8}$$

where m and b are as defined above, c is the asymptotic amount of growth as t increases indefinitely and e = 2.781.

The growth curve of *A. xylosoxidans*, OD versus time for each incubation temperature was fitted using Levenberg Marquardt nonlinear least-squares algorithm in Origin 2018 data analysis and graphing software (Originlab Corporation, Northampton, MA, USA) with 95 % confidence interval for all the parameters.

2.5 Model Performance

The statistical indicators that were used to compare the candidate models that describe the growth of *Achromobacter xylosoxidans* were; Coefficient of determination (R^2), Root Mean Square Error (RMSE), Aike's information criterion (AIC) and Shwarz Bayesian information criterion (BIC) and are mathematically expressed in form of Eqs(9), (10), (11) and (12), respectively.

$$R^2 = \frac{\sum_{i=1}^{n}(y - \hat{y})^2}{\sum_{i=1}^{n}(y - \bar{y})^2} \tag{9}$$

$$RMSE = \left[\frac{\sum_{i=1}^{n}(\hat{y} - y)^2}{n}\right]^{\frac{1}{2}} \tag{10}$$

$$AIC = n.\ln\left(\frac{RSS}{n}\right) + 2k \tag{11}$$

$$BIC = n.\ln\left(\frac{RSS}{n}\right) + k.\ln(n) \tag{12}$$

where n is the number of observations, y is the observed values at the i^{th}temperature $\hat{y}$ is the predicted values at the i^{th} temperature, $\bar{y}$ is the mean of the predicted values at the i^{th} temperature, RSS is the residual sum of squares in the model and k is the number of parameters in the model.

To select the best model between the two candidate models, a new weight which integrates the Eqs(9) to (12) was used and is expressed as Eq(13) (Longhi et al., 2013):

$$\alpha_i = \frac{\beta_i}{\sum_{k=i}^{M}\beta_k} \tag{13}$$

where α_i is the weighed mean of standardized indicators, i = 1, 2..M and β_i was computed based on Eq(14) (Longhi et al., 2013):

$$\beta_i = \frac{1}{4}\left[\frac{\left|R_i^2 - R_{min}^2\right|}{R_{max}^2 - R_{min}^2} + \frac{|RMSE_i - RMSE_{min}|}{RMSE_{max} - RMSE_{min}} + \frac{|AIC_i - AIC_{max}|}{AIC_{max} - AIC_{min}} + \frac{|BIC_i - BIC_{max}|}{BIC_{max} - BIC_{min}}\right] \tag{14}$$

where $(y)_{max}$represents the maximum y value in the two candidate models, $(y)_{min}$represents the minimum y value in the two candidate models y_i represents the y value of the i^{th} candidate model and i =1, 2,..M. In this work, R^2, RMSE, AIC and BIC were used to calculate β_i.

3. Results and discussion

The degradation of butyric acid in batch reactors led to the formation of biomass of *Achromobacter xylosoxidans*. The quantity of biomass formed increased with the degradation of butyric acid but increased exponentially with respect to incubation time during the log phase (data not shown.). Further, the increase in biomass concentration was dependent on the concentration of butyric acid remaining in the solution (data not shown). Numerous mathematical models that describe microbial growth in culture media have been and continue to be developed and used. Table 1 shows the mathematical parameters given by modified Gompertz and logistic models for the growth curves of *A. xylosoxidans* plotted with the mean OD values obtained from the experiments performed under different isothermal conditions. The actual and estimated growth curves of the two models are shown in Figure 1.

Table 1: Estimates of parameters and their standard errors for the studied growth models

Model	Mathematical parameter	Temperature			
		25 °C	30 °C	35 °C	40 °C
Logistic	a	1.314	1.331	1.340	1.144
		(0.166)	(0.083)	(0.160)	(0.031)
	b	5.294	5.523	4.184	3.940
		(0.175)	(0.061)	(0.116)	(0.010)
	c	0.365	0.486	0.295	0.253
		(0.134)	(0.110)	(0.085)	(0.023)
Gompertz	a	1.428	1.389	1.526	1.216
		(0.346)	(0.161)	(0.385)	(0.066)
	b	2.618	2.986	1.948	2.023
		(0.204)	(0.078)	(0.157)	(0.014)
	c	0.202	0.298	0.154	0.152
		(0.111)	(0.102)	(0.070)	(0.022)

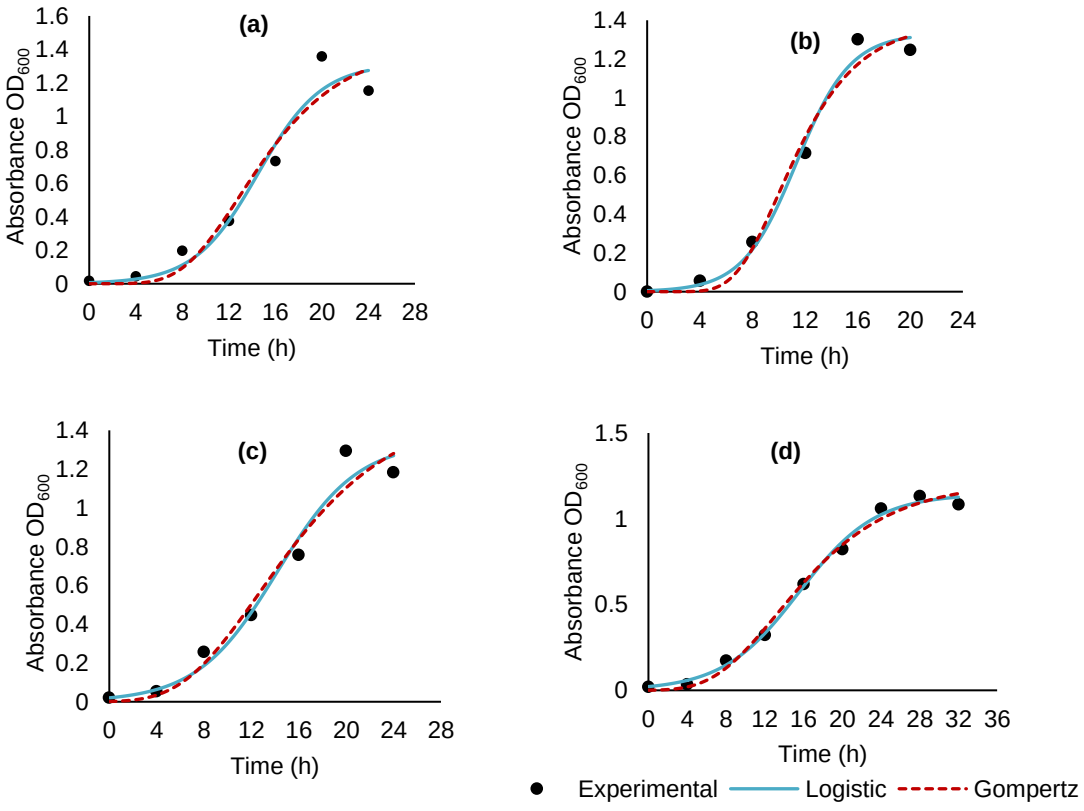

Figure 1: Growth curves of A. xylosoxidans by modified Gompertz and modified logistic model at different temperatures: (a) 25 °C, (b) 30 °C, (c) 35 °C and (d) 40 °C

It was observed that the growth patterns as is evident in Figure 1 were not identical at different set temperatures. The curves, however, showed good fit of the data by both the modified Gompertz and logistic models. The considerable advantage of modelling the experimental data using these models is that microbiologically meaningful parameters not purely mathematical concepts are obtained. Hence, the mathematical parameters obtained in Table 1 were used to determine the parameters of microbiological relevance as shown in Table 2. As shown in Table 2, the highest asymptotic value,A, was obtained at 35 ºC, whereas, the lowest was at 40 °C.

Table 2: Growth kinetic parameters of. A. xylosoxidans at different constant temperatures

Model	Microbiological parameter	Temperature			
		25 ºC	30 ºC	35 ºC	40 ºC
Logistic	A	1.314	1.331	1.340	1.143
	μ_{max}[h^{-1}]	0.120	0.162	0.099	0.072
	λ[h]	9.02	7.75	7.40	7.64
Gompertz	A	1.428	1.389	1.526	1.216
	μ_{max}[h^{-1}]	0.106	0.152	0.086	0.068
	λ[h]	8.01	6.66	6.16	6.73

The longest lag duration λ was obtained at 35 ºC whereas the shortest was at 40 ºC. The highest maximum specific growth rate,μ_{max}, was obtained at 30 ºC whereas the lowest was at 40 ºC. The results show that the estimated parameters of microbiological relevance, lag duration and maximum specific growth rate were higher for modified logistic model compared to modified Gompertz model.

These results are in contrast with the previous reports putting forward that the modified Gompertz model overestimate the two parameters (Baty and Delignette-Muller, 2004). However, Longhi et al., (2017) investigated the growth patterns of *Lactobacillus plantarum* at six isothermal conditions. They reported that the λ and μ_{max} obtained by modified logistic model presented higher values among the evaluated models *inter alia* modified Gompertz model, which is in agreement with the results of the current work. For the model selection criteria as shown in Table 3, R^2 and RMSE values for both models were found to between 0.944 and 0.996 and 0.055 and 0.118, respectively**.**

Table 3: Goodness-of-fit criteria for the growth models applied to the experimental data set of A. xylosoxidans

Model	Statistical indicator	Temperature			
		25 ºC	30 ºC	35 ºC	40 ºC
Logistic	R^2	0.959	0.989	0.972	0.996
	RMSE	0.101	0.055	0.081	0.067
	AIC	-26.07	-28.68	-29.20	-57.77
	BIC	-26.23	-29.31	-29.37	-57.18
Gompertz	R^2	0.944	0.980	0.962	0.992
	RMSE	0.118	0.074	0.093	0.039
	AIC	-23.97	-25.26	-27.29	-52.38
	BIC	-24.13	-25.88	-27.45	-51.79

These high R^2 values suggest that the fitting of both primary models to the experimental growth data were very good. However, it is reported in literature that do not show the goodness-of-fit of the non-linear models (Shi and Ge, 2010), hence, other statistical indices AIC and BIC for good comparison. As shown in Table 3, BIC and AIC values obtained were between -57.18 and -24.13 and -57.77 and -23.97, respectively. According to the higher values of R^2 and lower values of RMSE, AIC and BIC obtained in this work, the modified logistic model was determined to be the best fitting model to the experimental growth data of *A. xylosoxidans.* Fujikawa and Morozumi (2006) also reported that the modified logistic model successfully described growth curves of *Escherichia coli* and *Salmonella* as compared to the Gompertz model. To reaffirm the obtained results based on R^2, RMSE, AIC and BIC values the new weight,α_i, revealed that the modified logistic model provided the best fit to the growth data which was 1.5 times (0.6) better in comparison to modified Gompertz model (0.4).

4. Conclusions

This study has clearly demonstrated that the growth kinetic modelling could be a useful tool for predicting butyric acid biodeodorisation processes. The experimental growth data in liquid medium containing butyric acid as a sole source of carbon at various isothermal conditions were successfully fitted with the two primary models. However, the modified logistic model provided the best fit for the experimental growth data. Further, since the parameters of microbiological meaning were estimated based on the numerical parameters obtained from the

primary models, the microbiological parameters obtained based on the modified logistic model are suitable for expressing the growth curves of *A.xylosoxidans* using butyric acid as a sole source of carbon. The variability in maximum growth rates found at different constant temperatures emphasises the need to take into account the implications of temperature in biodeodorisation processes using *A.xylosoxidans*. Collectively, the ability to predict the growth of *A.xylosoxidans* as a function of incubation temperature will contribute to the development of secondary and tertiary models that can be used to predict the effect of temperature on the microbial response in liquid medium under non-isothermal conditions. This study will also contribute to efforts to optimise the biodeodorisation of butyric acid emitted from pit latrines for their increased adoption and consistent usage.

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