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Hyperbolastic Models to Describe Yeast Growth Curves in Batch Ethanol Fermentation

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ABSTRACT: *Sigmoidal models were compared to describe the growth curve of Saccharomyces cerevisiae in batch ethanol fermentation of sugarcane molasses. Five classical functions and some of their reparameterizations were fitted to three experimental data sets: logistic, Gompertz, Chapman-Richards, Morgan-Mercer-Flodin and a Weibull-type model. Since these models are nonlinear, measures of nonlinearity were used to evaluate the statistical properties of the least squares estimators. The measures used were the intrinsic (IN) and parameter-effects (PE) curvatures of Bates and Watts, the bias measure of Box, and the Hougaard's measure of skewness. Among the models analyzed, only a reparameterization of the Weibull-type model presented close to linear behavior, ensuring the statistical validity of the parameters estimated by the method of least squares.*

KEYWORDS*: Saccharomyces cerevisiae, ethanol fermentation, hyperbolastic models, measures of nonlinearity.*

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I. INTRODUCTION

Saccharomyces cerevisiae growth

Saccharomyces cerevisiae, commonly known as baker's yeast, is popular and commercially significant among the yeasts. This microorganism has been used for a long time in the food industry, mainly in the baking, and alcoholic beverages industries. The Saccharomyces cerevisiae has also been used to produce ethanol by using fermentation of sugarcane juice or molasses, and more recently, by fermentation of lignocellulosic biomass, which further increases the importance of this microorganism in the biofuel industry.

Yeast cells grow in three main phases: lag, exponential, and stationary. When a culture of yeast cells is inoculated in a different growth medium, it enters the lag phase or adaptation time. In this phase, the cells are biochemically active, but they do not divide. After this, the cell growth increases, initially, slowly, in a positive acceleration phase; then it increases rapidly, approaching an exponential growth rate; but, after this rapid growth, the acceleration declines in a negative phase until a zero-growth rate, when the population stabilizes. This slowdown in the growth rate results in an environmental resistance increase, which becomes proportionately more important at a higher density of cell's population. This type of growth is termed "density-dependent", since the growth rate depends on the number of cells available in the population. The point of stabilization, or zero-growth rate, is termed the "saturation value" or "carrying capacity" of the environment for that microorganism (Allaby, 2014). Moreover, if the cell concentration is plotted against time, a typical sigmoidal or "S-shaped" growth curve is obtained.

Processes producing sigmoidal or "S-shaped" growth curves are widespread in biology, agriculture, engineering, and economics. Such curves start at some fixed point and increase their growth rate monotonically to reach an inflexion point. After this, the growth rate decreases to approach asymptotically some final value. Additionally, numerous mathematical functions have been proposed for modeling sigmoidal growth curves, many of which are claimed to have some underlying theoretical bases. Among these are the logistic equation and the Gompertz model.

Hyperbolastic models

Tabatabai et al. (2005) developed three sigmoid growth models called "hyperbolastic models" for predicting and analyzing self-limited growth behavior. These models are briefly presented below. For further details, see the original work.

Hyperbolastic model H1

The following function is called hyperbolastic growth model of type I or simply H1.

$$P(t) = \frac{M}{1+\alpha . exp\,[-M\beta t - \theta \operatorname{arcsinh}(t)]} \quad (1)$$

Where P(t) represents the population size at time t, β is the parameter representing the intrinsic growth rate, α and θ are parameters, and M represents the maximum sustainable population (carrying capacity), which is assumed to be constant, though, in general, the carrying capacity may change over time. For growth curves, β must be positive, leading to an eventually increasing curve with an asymptote at M; β can be negative only for eventual inhibition curves or decay. If θ = 0, then the equation (1) reduces to a general logistic model.

The observed value of P(t) at t = 0 is used to obtain an approximate value of α:

$$\alpha = \frac{M-P(0)}{P(0)} exp[M\beta t_0 + \theta \operatorname{arcsinh}(t_0)] \quad (2)$$

Replacing equation (2) in equation (1), the H1 model becomes a three-parameter model.

Hyperbolastic model H2

The function P(t) in equation (3) is called hyperbolastic growth model of type II or simply H2.

$$P(t) = \frac{M}{1+\alpha . \operatorname{arcsinh}(t)[\exp(-M\beta t^{\gamma}]} \quad (3)$$

Where M is the carrying capacity, and α, β and γ are parameters. As in the H1 model, the observed value of P(t) at t=0 is used to obtain an approximate value of α:

$$\alpha = \frac{M-P(0)}{P(0) arcsin\,h[exp\,(-M\beta\, t_0^{\gamma})]} \quad (4)$$

Replacing equation (4) in equation (3), the H2 model becomes a three-parameter model.

Hyperbolastic model H3

The function in equation (5) is called hyperbolastic growth model of type III or simply H3.

$$P(t) = M - \alpha . exp[-\beta t^{\gamma} - \operatorname{arcsinh}(\theta . t)] \quad (6)$$

Again, M is the carrying capacity and α, β, γ and θ are parameters If θ = 0, then this model reduces to the Weibull function. As in the H1 and H2 models, the observed value of P(t) at t=0 is used to obtain an approximate value of α:

$$\alpha = (M - P(0)) exp\left[\beta t_0^{\gamma} + \operatorname{arcsinh}(\theta t_0)\right] \quad (7)$$

Replacing equation (7) in equation (6), the H3 model becomes a four-parameter model.

The aim of this work was to compare the hyperbolastic models with the classic growth models, in the description of the growth curve of Saccharomyces cerevisiae during ethanol production. Since these models are nonlinear, measures of nonlinearity were used to evaluate the statistical properties of the least-squares estimators. The measures used were the intrinsic (IN) and parameter-effects (PE) curvatures of Bates and Watts, the bias measure of Box, and the Hougaard's measure of skewness.

Measures of nonlinear behavior

Most asymptotic inferences for nonlinear regression models are based on analogies with linear models. Since these inferences are approximate, some measures of nonlinearity have been proposed as a guide for understanding how good linear approximations are likely to be (El-Shaarawi&Piegorsch, 2002). The most used measures of nonlinearity are the curvature measures of Bates and Watts (1980), the bias measure of Box (1971), and the Hougaard's (1985) measure of skewness. These measures are described summarized below. For further details, see the original works.

Curvature measures

Bates and Watts (1980) divide the concept of nonlinearity into two parts: intrinsic nonlinearity (IN) and parameter-effects nonlinearity (PE). Relative intrinsic and parameter-effects curvatures can be used to quantify the global nonlinearity of a nonlinear regression model. Furthermore, the intrinsic nonlinearity (IN) measures the curvature of the solution locus in sample space. For a linear regression model, IN is zero since the solution locus is straight (a line, plane, or hyperplane). For a nonlinear regression model, the solution locus is curved, with IN measuring the extent of that curvature (Ratkowsky, 1990). The parameter-effect nonlinearity (PE) is a measure of the lack of parallelism and the inequality of spacing of parameter lines on the solution locus at the least-squares solution. (Bates & Watts, 1980).

If IN and PE can be considered small, the nonlinear model behaves close to a linear model, i.e., the least squares estimators of the parameters are close to being unbiased and normally distributed, and they have minimum variance. Ratkowsky (1983) termed such nonlinear regression models close-to-linear models. The criteria that determine if IN and PE are small enough (or not statistically significant) so that the nonlinear model can be considered close-to-linear are presented in section Matherial and methods: curvature measures.

Bias and skewness

In practice, only a few of the parameters might dominate the global nonlinearity. These parameters are the reparameterization parameters of interest. Unfortunately, the global nonlinearity measures of Bates and Watts (1980) do not differentiate the parameters based on their contribution to the overall curvature. Additionally, manifestations of nonlinear behavior include significant bias and skewness. Hence, it is essential to estimate, at least these, basic statistical properties of the parameter estimates, in order to identify the reparameterization parameters of interest (Gebremariam, 2014).

The bias and skewness of the parameter estimates of a nonlinear regression model can be estimated by using the bias measure of Box (1971) and the Hougaard (1985) measure of skewness. Box's bias represents the discrepancy between the estimates of the parameters and the true values. Skewness is a measure of lack of symmetry. Hougaard's measure of skewness can be employed to assess whether a parameter is close to linear or if it contains considerable nonlinearity, because of the close link between the extent of nonlinear behavior of an estimator, and the extent of nonnormality in the sampling distribution of this estimator (Ratkowsky, 1990).

II. MATERIAL ANDMETHODS

Strain, medium, fermentation and analyses

A strain of Saccharomyces cerevisiae (Mauriferm Y904, Mauri, Brazil) was cultivated in two distinct fermentation media: diluted sugar cane molasses, and a synthetic medium containing 3g/L of bacteriological peptone, 5 g/L of yeast extract and 20 g/L of glucose. The inoculum preparation included the suspension of 100 g of the dried yeast in 900 g of water, maintained in a shake flask at 100 rpm and 34 °C for 30 minutes. The experiments were carried out in duplicate at 34 ºC and 100 rpm in a 3.0 L fermentor NBS Bioflo 110 (New Brunswick Scientific, USA), in which 2000 mL of the culture medium was inoculated with 1% of seed culture. Samples were withdrawn at regular time intervals and measurements of the dry weight of cells quantified the biomass concentration.

Models

The models fitted to the experimental growth data of Saccharomyces cerevisiae are shown in Table I. In H1, H2 and H3 models (see Equations 1, 3 and 6), the variable P(t) was replaced by X(t), where X(t) is the biomass concentration at time t.

The fits of the hyperbolastic models to the experimental data were compared with those of the logistic, Weibull and Gompertz models. These classical sigmoid models were not chosen at random. The hyperbolastic models H1 and H3 (Equations 1 and 5) reduce to the logistic and Weibull models, respectively, when $\theta = 0$. The Gompertz model was included as an alternative to the logistic model because has an asymmetric inflection point and a shorter period of fast growth.

Table 1

Sigmoidal models fitted to the Saccharomyces cerevisiae growth data.

Model	Equation
H1	$X(t) = \frac{M}{1+\alpha.exp[-M\beta t - \theta \text{arcsinh}(t)]}$, where $\alpha = \frac{M-X(0)}{X(0)} exp[M\beta t_0 + \theta \text{arcsinh}(t_0)]$
H2	$X(t) = \frac{M}{1+\alpha.\text{arcsinh}(t)[\exp(-M\beta t^{\gamma}]}$, where $\alpha = \frac{M-X(0)}{X(0) arcsinh[exp(-M\beta t_0^{\gamma})]}$
H3	$X(t) = M - \alpha.exp[-\beta t^{\gamma} - \text{arcsinh}(\theta.t)]$, where $\alpha = (M - X(0))exp[\beta t_0^{\gamma} + \text{arcsinh}(\theta t_0)]$
Logistic	$X(t) = \delta + \alpha / (1 + \exp(\beta - \gamma t))$
Gompertz	$X(t) = \delta + \alpha.\exp(-\exp(\beta - \gamma t)$
Weibull	$X(t) = \alpha - \beta\exp(-\gamma t^{\delta})$

Measures of nonlinearity

In this study, the parameter estimates and their respective measures of nonlinearity were obtained by using the NLIN procedure of the SAS software. For each measure, the criteria for assessing the extent of nonlinear behavior of the parameter estimates are described below. Details about the development, procedure, and equations for determining the curvature measures of nonlinearity of Bates and Watts (1980), the bias measure of Box (1971), and the Hougaard (1985) measure of skewness are found in the original works.

Curvature measures

The statistical significance of the intrinsic nonlinearity (IN) and the parameter-effects nonlinearity (PE) were evaluated by comparing these values with$1/\sqrt{F}$, where $F = F(\alpha, n-p, p)$is the inverse of Fisher´s probability distribution obtained at significance level α=0.05, p is the number of parameters and n is the number of observations. The value $1/\sqrt{F}$ may be regarded as the radius of the curvature of the $100(1-\alpha)\%$ confidence region. Hence, the solution locus may be considered to be sufficiently linear within an approximately 95% confidence region if IN $<1/\sqrt{F}$ (α= 0.05). Similarly, if PE $<1/\sqrt{F}$ the projected parameter lines on the solution locus may be regarded as being sufficiently parallel and uniformly spaced, i.e., the parameter estimates do not depend on the user being able to supply a good initial estimate and the tests of parameters invariance will be adequate. (Ratkowsky, 1990).

Box's bias

Box's bias in the least square estimates of the parameters in nonlinear regression can be expressed as a percentage of the least square estimate. This percentage bias is estimated by:

$\%Bias(\hat{\theta}) = 100.Bias(\hat{\theta})/(\hat{\theta})$ (8)

Where $\hat{\theta}$ is the least square parameter estimate. According to Ratkowsky (1983), a percentage bias greater than 1% in absolute value is considered to be significantly nonlinear.

Hougaard's skewness

The degree to which a parameter estimator exhibits nonlinear behavior can be assessed with Hougaard's measure of skewness, g1i. According to Ratkowsky (1990), if |g1i| < 0.1, the estimator of the parameter is very close to linear and, if 0.1 < |g1i| < 0.25, the estimator is reasonably close to linear. For |g1i| > 0.25, the skewness is very apparent, and |g1i| > 1 indicates considerable nonlinear behavior.

III. RESULTS AND DISCUSSION

Table II shows the results of the growth of *Saccharomyces cerevisiae*.

Table 2

Dry mass concentration (X) of Saccharomyces cerevisiae in the two fermentation media. Each data set corresponds to the arithmetical mean of two runs.

Sugarcane molasses		Synthetic medium	
t (h)	**X (g/L)**	**t (h)**	**X (g/L)**
0	0.18	0	0.32
4	0.46	1	0.52
8	1.09	2	0.96
12	2.27	3	1.48
16	3.73	4	1.88
20	4.91	5	2.10
24	5.54	6	2.26
28	5.82	7	2.28

Measures of nonlinearity

The models in Table 1 were fitted to the experimental results shown in Table 2. Tables 3 and 4 show the least squares parameter estimates with the respective values for the measures of nonlinearity to the two experimental data sets.

For all models, IN $<1/\sqrt{F}\ (\alpha = 0.05)$ and therefore the solution locus may be considered to be sufficiently linear within an approximately 95% confidence interval. However, only the logistic model presented PE $<1/\sqrt{F}$, indicating that the projected parameter lines on the solution locus may be regarded as being sufficiently parallel and uniformly spaced. Therefore, among the studied models, the logistic model was the only one that presented close-to-linear behavior, ensuring the statistical validity of the parameters estimated by the method of least squares. For this model, the parameter estimates are almost unbiased, normally distributed and have close-to-minimal variance.

On the other hand, Gompertz and Weibull models, and all the hyperbolastic models have exhibited high parameter-effects curvature (PE $>1/\sqrt{F}$). This indicates that at least one parameter in these models is departing from linear behavior, and the Hougaard's skewness and Box's bias indicate which parameter or parameters are responsible for this behavior. For the H1 model, measures of skewness and bias indicate that all parameter estimates in Table 3 can be considered close-to-linear. However, the parameter-effects curvature exceeded the critical value. In this case, the nonlinear behavior is probably due to the estimate of M, that presented the highest skewness among the parameter estimates. In Table 4, all parameter estimates for this model are skewed and biased.

Table 3

Statistical results of the least-squares estimation for the models in Table 1. Medium: diluted sugarcane molasses.

Model	IN	PE	ρ	Parameter	Estimate	Std. Error	Skewness	% Bias
H1	0.039	0.550	0.430	M	6.047	0.066	0.16	0.04
				β	0.036	0.002	0.07	0.10
				θ	0.342	0.042	-0.07	-0.22
H2	0.036	1.258	0.390	M	6.119	0.076	0.19	0.05
				β	0.092	0.005	0.04	-0.02
				γ	0.774	0.023	0.07	0.05
H3	0.072	24.039	0.331	M	5.849	0.039	0.26	0.44
				β	0.789	0.123	1.08	6.58
				γ	3.063	0.128	0.07	0.08
				θ	0.013	0.002	-0.18	-0.47
Logistic	0.004	0.020	0.430	α	5.996	0.006	0.01	$1.9 \cdot 10^{-5}$
				β	3.498	0.008	0.01	$6 \cdot 10^{-4}$
				γ	0.25	$7 \cdot 10^{-4}$	0.01	$6 \cdot 10^{-4}$
Gompertz	0.175	1.149	0.430	α	6.580	0.291	0.49	0.34
				β	1.712	0.134	0.37	0.61
				γ	0.142	0.014	0.23	0.55
Weibull	0.016	3.818	0.331	α	-5.540	0.034	-0.06	0.01
				β	$5 \cdot 10^{-4}$	$5 \cdot 10^{-5}$	0.27	0.42
				γ	2.720	0.036	0.02	0.01
				δ	5.883	0.021	0.08	0.01

ρ = critical curvature value ($\rho = 1/\sqrt{F}$), F (α, n-p, p) is the inverse of Fisher´s probabilitydistribution obtained at significance level α = 0.05, p is the number of parameters and n is the number ofobservations.

Table 4

Statistical results of the least-squares estimation for the models in Table 1. Synthetic medium.

Model	IN	PE	ρ	Parameter	Estimate	Std. Error	Skewness	% Bias
H1	0.334	22.060	0.430	M	2.485	0.404	6.55	18.4
				β	0.072	0.221	-0.89	-34.8
				θ	1.768	0.729	0.93	4.73
H2	0.058	0.793	0.390	M	2.420	0.054	0.42	0.17
				β	0.831	0.022	0.04	-0.23
				γ	0.601	0.035	0.10	0.16
H3	0.182	14.930	0.331	M	2.294	0.028	0.51	0.13
				β	0.024	0.012	1.63	13.4
				γ	2.667	0.349	0.02	0.30
				θ	0.189	0.025	-0.75	-1.84
Logistic	0.052	0.259	0.430	α	2.343	0.029	0.12	0.04
				β	2.038	0.069	0.12	0.11
				γ	0.852	0.034	0.11	0.10
Gompertz	0.094	1.111	0.430	α	2.507	0.108	0.52	0.40
				β	0.911	0.087	0.33	0.80
				γ	0.514	0.058	0.21	0.61
Weibull	0.068	2.37	0.331	α	-1.976	0.054	-0.31	0.17
				β	0.099	0.013	0.37	0.69
				γ	1.970	0.105	0.02	0.07
				δ	2.307	0.021	0.29	0.06

ρ = critical curvature value ($\rho = 1/\sqrt{F}$), F (α, n-p, p) is the inverse of Fisher´s probabilitydistribution obtained at significance level α = 0.05, p is the number of parameters and n is the number ofobservations.

The estimates of M for the H2 model are skewed and therefore responsible for the significant parameter-effects curvature. For the H3 model, the high parameter-effect curvature is due to the estimates of M and β. The estimates of M are skewed and the estimates of β are skewed and biased.

All the parameter estimates of the Gompertz model are skewed and therefore responsible for the significant parameter-effects curvature. For the Weibull model, the nonlinear behavior is due to the high skewness of β (Table 3) and α estimates (Tables 3 and 4).

The fit of the logistic model to each data set is shown in Figure 1. The other models presented far from linear behavior, which implies invalid inference results based on asymptotic approximations for the least squares estimators. Therefore, it does not make sense to display graphs representing the fit of these models to the experimental data.

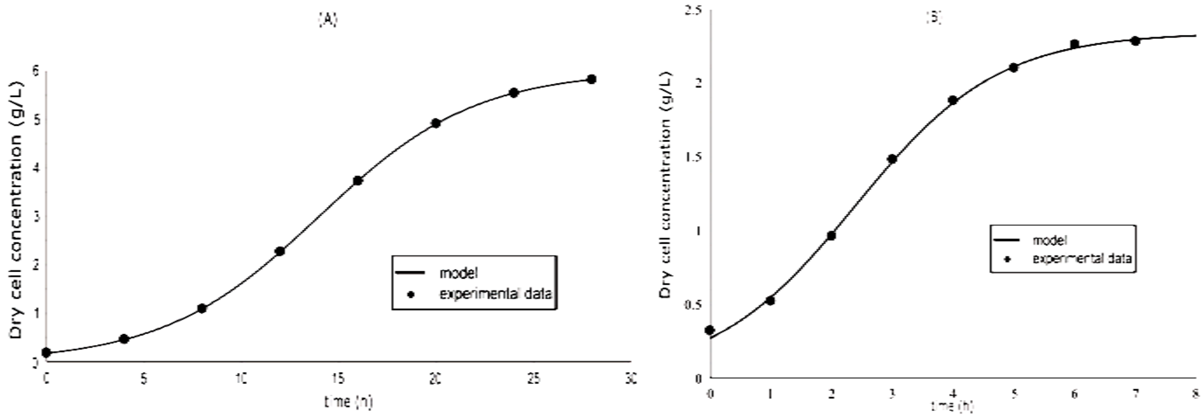

Figure 1. Growth curve of *Saccharomyces cerevisiae* fitted with the logistic model.
(A) Sugarcane molasses; (B) Synthetic medium.

IV. CONCLUSION

In this work, hyperbolastic and classical sigmoid models were compared to describe the growth curve of Saccharomyces cerevisiae in batch ethanol fermentation. Among all the models fitted to the experimental data, only the logistic model presented a close-to-linear behavior, ensuring the statistical validity of the parameters estimated by the method of least-squares. For this model, the parameter estimates are almost unbiased, normally distributed and have close-to-minimal variance.

The hyperbolastic models, in addition to Gompertz and Weibull models presented a far-from-linear behavior, due to skewed or biased parameter estimates, which implies invalid inference results based on asymptotic approximations for the least-squares estimators.

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