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Modeling the growth of *Pseudomonas putida* using the Bertalanffy-Pütter model .

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ABSTRACT

Anadurai et al. (2000) fitted the models of Verhulst (logistic growth), Gompertz and Richards to given growth data of *Pseudomonas putida*. From the three models tested, the logistic model fitted best. Each of the forementioned models corresponds to a specific exponent-pair of the five parameter Bertalanffy-Pütter$BP(a, b)$ model class. We found that the sum of squared errors (SSE) of the model $BP(2.81, 3.32)$ improved the SSE of the logistic model $BP(1, 2)$ by the factor of five. Furthermore, assuming a constant biomass-substrat-yield, the best-fit BP model had a slightly better fit than the classical Monod model.

Keywords: batch microbial growth, Bertalanffy-Pütter growth models, Monod model, *Pseudomonas putida*, simulated annealing

Abbreviations: AIC, Akaike information criterion; **SSE**, sum of squared errors between the model curve and the data; **BP-model**, Bertalanffy-Pütter type growth model

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INTRODUCTION

Microbial growth is traditionally described by Monod's (1949) kinetics with multiple applications in biotechnology (e.g. Steele et al., 1977, Kyriakopoulos et al., 2017). Recent literature identified also more elaborate alternatives to the Monod model (e.g. Tsipa et al., 2018, Wade et al., 2016). Mathematically, the Monod kinetics is a system (1) of differential equations that relates the growth rate of biomass concentration $x(t)$ at time t to the rate-limiting substrate concentration $s(t)$, using five positive model parameters, the maximum specific growth rate μ_{max}, the saturation constant k, the yield coefficient Y, and the initial conditions $x(0) = x_0$, $s(0) = s_0$.

$$x'(t) = \frac{\mu_{max} \cdot s(t)}{k + s(t)} \cdot x(t) \text{ and } s'(t) = -x'(t)/Y \tag{1}$$

As the saturation constant cannot be determined experimentally, it is estimated from solutions of (1) with the best fit to growth data (Shirsat et al., 2015; Liu, 2007). If there are no data about the substrate concentration, the model (1) is reformulated as a differential equation (2) for biomass concentration alone (Shuler & Kargi, 2002), using the five non-negative parameters α, β, γ, δ (related to the above parameters) and x_0.

$$x'(t) = \frac{\alpha - \beta \cdot x(t)}{\gamma - \delta \cdot x(t)} \cdot x(t) \tag{2}$$

Further, various simpler models (fewer parameters) have been considered in literature to describe the growth of biomass and thereby approximate the Monod model. Examples are Verhulst (1838) logistic growth, Gompertz (1832) and Richards (1959) models (e.g. Annadurai et al., 2000; Arora et al., 2018; Kargi, 2009). These models can be unified and generalized by the Bertalanffy-Pütter model (BP-model), which is defined by differential equation (3) of Pütter(1920). (For another generalization c.f. Tsoularis & Wallace, 2002.) It uses five parameters: the exponent-pair $0 \le a < b$, constants p and q, and the initial concentration $x(0) = x_0 > 0$. In general, its solution needs non-elementary functions (Marusic & Bajzer, 1993; Ohnishi et al., 2014).

$$x'(t) = p \cdot x(t)^a - q \cdot x(t)^b \tag{3}$$

We interpret equation (3) as a definition of a model class, as each exponent-pair defines a different growth model $BP(a, b)$ with three parameters (p, q and x_0). Several models that have been used previously to describe growth are of this form, e.g. the Brody (1945) model of bounded exponential growth $BP(0, 1)$, logistic growth $BP(1, 2)$, the model $BP(2/3, 1)$ of von Bertalanffy (1949), or the model $BP(3/4, 1)$ of West et al. (2001). Also, the Gompertz model fits into this scheme: It is the limit case (with a different differential equation), where b converges to $a = 1$ from above (Marusic & Bajzer, 1993). Equation (3) also includes several classes of models, such as the Richards model ($a = 1$ with b variable) and the generalized Bertalanffy model ($b = 1$ with a variable; in economy this is theSolow-Swan growth model). In animal growth, the latter model classes were considered, as no single exponent-pair could provide a satisfactory description of growth (Pauly, 1981; White, 2010).
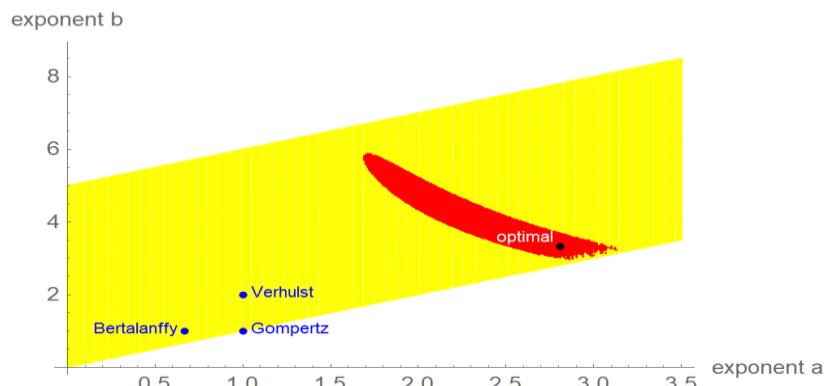

Figure 1. Optimization of model 3: grid points (yellow), optimal exponent-pair (black), Bertalanffy, Gompertz and Verhulst (logistic) exponent-pairs (blue), near-optimal exponent-pairs (red).

When compared to the range of models considered for this paper (yellow area), these named models and model-classes appear as rather exceptional (Figure 1).

We therefore ask, by how much the fit to the data could be improved by using the BP-class (3) of models instead of the above special cases. In previous papers, we have asked this question for modeling the growth of tumor (Kühleitner et al., 2019a), wild-caught fish (Renner-Martin et al., 2018), chicken (Kühleitner et al., 2019b), or dinosaurs (Brunner et al., 2019), and we found substantial improvements in the fit by using the BP-model class. Here we use data from Annadurai et al. (2000) about the growth of *Pseudomonas putida* and determine the best fits to the general BP-class (3) and also to the Monod model (2). Pseudonomas strains have multiple applications in biotechnology, e.g. degradation of water pollutants (García-Uitzet al., 2016), whence there is some interest in determining their growth pattern.

MATERIALS AND METHODS

Software

The data were processed in Mathematica 11.3 of Wolfram Research® and the output of optimization was exported to a spreadsheet that is provided as a supporting material.

Data

The paper uses the data from Table 1 of Annadurai et al. (2000). It recorded N= 27 hourly measurements (time t = 1, 2, …, 27) of OD_{600} (optical density at a wavelength of 600 nm) for a batch culture of *Pseudomonas putida* (Trevisan, 1889) subsequent to the lag-phase; see the source paper for a detailed documentation of the experiment.

We decided to use these data, as they display a sigmoidal shape and as the number of time points is exceptionally large, whence it becomes easier to distinguish good and bad fitting models. The data are relatively old, but we do not expect that more recent data would display a fundamentally different growth pattern. The source did not record statistical information (e.g. error-bars) and such information is not needed for this paper.

Data-fitting

We used the least-squares method; this means that the hypothesis of a normal distribution of errors was assumed. For equation (3) this led to an optimization problem that previously was almost intractable. Recently, in Renner-Martin et al. (2018) we succeeded in developing an advanced optimization method, based on simulated annealing (Vidal, 1993). The optimal exponent-pairs were searched on a grid with an accuracy of 0.01, and for each grid point the other parameters p, q, x_0 were found by means of simulated annealing. If the best-fit exponents were on the boundary of the search grid, more grid-points were considered to guarantee optimality. Furthermore, the paper visualizes the near-optimal exponents, whose best-fit *SSE* did not exceed the least value of *SSE* by 5% or more. For the Monod-model (2) simulated annealing was applied to find all five optimal parameters.

Statistical analysis

As we consider one dataset for which we compared the fit by different models, we use the Akaike (1974) information criterion as tool from statistics. If *SSE* is the sum of squared errors, N is the number of data and K the number of model parameters, then equation (4) defines *AIC* (Burnham & Anderson, 2002; Motulsky & Christopoulos, 2003).

$$AIC = N \cdot \ln\left(\frac{SSE}{N}\right) + 2 \cdot K \tag{4}$$

For example, the Monod and Bertalanffy-Pütter models have the same number K = 6 of parameters (the five explicit parameters plus *SSE* as an additional parameter), while for Verhulst logistic growth K = 4. For our data, N = 27.

The model with the least *AIC* is best supported by the data (it should be selected). This is quantified by the following probability (Akaike weight): Based on *AIC*, equation (5) computes the probability that a model with *AIC* is true, when compared to the the model with least AIC_{min}:

$$prob = \frac{e^{-\Delta/2}}{1+e^{-\Delta/2}}, \text{ where } \Delta = AIC - AIC_{min} > 0 \qquad (5)$$

RESULTS

For the optimization of the BP-model, Figure 1 plots the grid points (almost 100,000 grid points were searched), the optimal exponent-pair, and the region of near-optimal exponent-pairs. For its optimal exponent-pair *a* = 2.81, *b* = 3.32 the least *SSE* was 0.00219 (other parameters: *p* = 1.682, *q* = 2.084, x_0 =0.212). As this exponent-pair was an inner point of the region of near-optimal exponent-pairs, this confirmed local optimality.

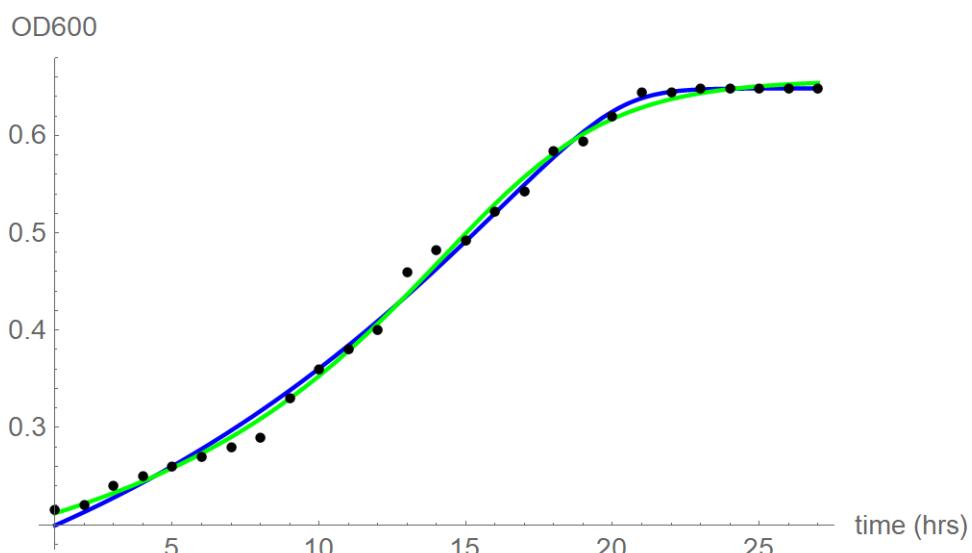Figure 2 plots the data and the best-fitting solutions of (3) and (2), respectively.

Figure 2. Data (black dots) and the best-fitting model curves for equations (3) and (2) in green and blue, respectively.

The BP(2.81, 3.32)-model achieved the best fit. For the near-optimal exponent-pairs, the best-fit model curves had barely a visible difference to the optimal model curve. The Monod model had a *SSE* of 0.00295 (other parameters: α = 0.231, β =0.357, γ = 3.381, δ = 4.931, x_0 = 0.199). This *SSE* was by ca. 35% higher than for the BP-model. Further, for the logistic model *SSE* was 0.01225 (*a* = 1, *b* = 2, *p* = 0.13, *q*= 0.171, x_0 = 0.178); this was about 5.6 times the *SSE* of the best-fit BP-model.

The *AIC*-values for the Verhulst, Monod and Bertalanffy-Pütter models were −199.8, −234.3, and −242.3, respectively. Thus, the Bertalanffy-Pütter model had the least *AIC*. When compared to this model, for Verhulst logistic growth and for the Monod kinetics the probabilities were 0% and 1.8%, respectively, that these models were true for the considered data.

DISCUSSION

For the present data the logistic model had the best fit amongst the models considered by Annadurai et al. (2000). As the logistic model is a special case of the BP-model (3) with more parameters, from an optimization of (3) we expected a slight improvement of this fit. Instead, we arrived at an exponent-pair that was far remote from the exponent-pairs of the logistic model and other models used in traditional growth studies; using this exponent-pair resulted in a substantial improvement of the fit.

As the Monod model is the traditional model for the growth of biomass in a batch, we expected another slight improvement for this model. Instead, whereas on a visual inspection the fit by the Monod model appeared to be satisfactory, the best-fit BP model had a significantly better fit than the Monod model, when the *AIC* criterion and its probability to be true were used. The reason for strong support for the BP-model was the large number of data points. A biological reason for the good fit of BP-models may be the tendency of *Pseudonomas putida* to form clusters. For clusters the biophysical reasoning in support of model (3) might apply, according to which the flow of substrates through the surface area of the cluster would be the major barrier to growth (Pauly, 1981; von Bertalanffy 1957).

Mathematically, the better fit of the BP-model was achieved by a different pattern of growth (e.g, no plateau phase) that deviated from the traditional picture for the Monod kinetics of growth in different phases. In order to visualize this difference between the models, Figure 3 compares the specific growth rates $\mu(t) = x'(t) / x(t)$.
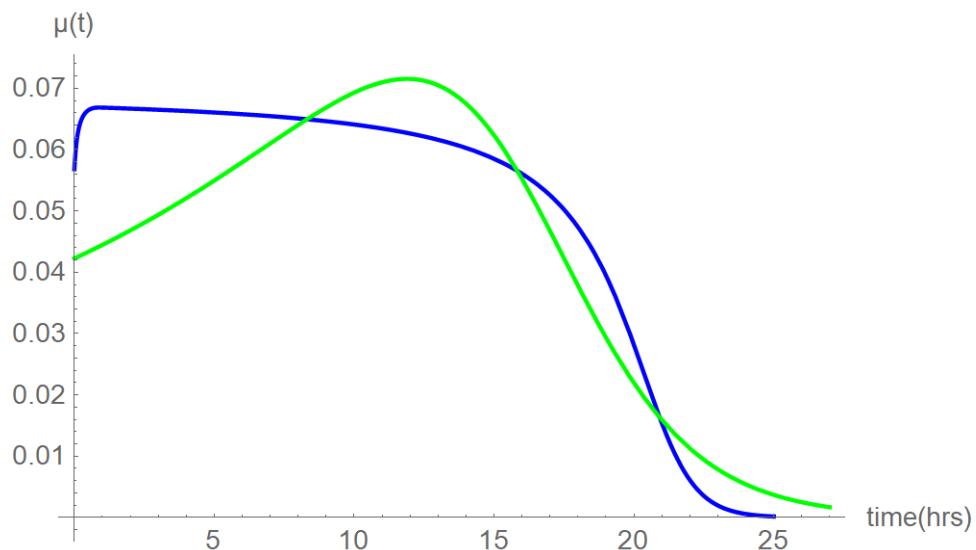

Figure 3. Specific growth rates for the best fitting growth curves for equations (3) and (2) in green and blue, respectively.

For the BP-model there was a long acceleration phase till the maximum specific growth rate $\mu = 0.073$ (1/hrs) was reached after 12.2 hours. It was followed by a deceleration phase, but a plateau phase or a stationary phase could not be discerned. For the Monod model, after a short acceleration phase the maximum specific growth rate of $\mu = 0.063$(1/hrs) was reached, followed by a plateau phase (exponential growth), a phase of deceleration, and the stationary phase (no growth).

Finally, we note that the best-fit parameters depend strongly on the method of calibration. For illustration, we repeated all computations using the lognormal distribution (meaning a higher variance for higher concentrations); this was equivalent to identifying the least squares fit of the logarithms of the model curves to the logarithms of the data. The best-fit BP-model (3) had the parameters a = 3.09, b = 3.25, p = 6.516, q= 6.974, x_0 = 0.214, and the best-fit Monod model (2) had the parameters α = 0.216, β = 0.335, γ = 3.404, δ = 5.139, x_0 = 0.205. Again, the BP-model had a better fit and also its *AIC* was least.

We conclude that the BP-model is a viable alternative for describing the growth of biomass. Where the BP-model has a better fit than the other named models considered in bioprocess engineering literature, this model may enable a more economic control of biotechnical processes.

Supporting information

We provide an additional file that contains a table of the used data and the optimization results (computation *of SSE$_{opt}$(a, b)* for certain grid points):

S1 File.xlsx. Sheet 1: table of the data (time in hours, optical density at wavelength 600nm), Sheet 2: table of the optimization results (exponent a, exponent b, and for them the best-fit parameters initial value of OD600, p, q, and SSE)

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