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GROWTH MODELS OF *THERMUS AQUATICUS* AND *THERMUS SCOTODUCTUS*

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Abstract

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Members of the genus *Thermus* family are signifiant producers of secondary metabolites, which are very commonly used in industry. Beside the productivity, it is also important to study the growth curve of each strain. The mathematical models which are commonly used to describe behavior of microbial strains under different physical and chemical conditions can reduce measured data.

In this study, the mathematical models which describe only the microbial count were used. For analysis, Verhulst model, von Bertalanffy model and Richards model were chosen.

During the cultivation of *Thermus aquaticus* and *Thermus scotoductus* strains, optical density (OD) and concentration of microorganisms were measured. The mathematical models were fitted to experimental data. The fitting was made in program MATLAB. The coefficients of models and statistical evaluation of goodness of fit of models were identified from mathematical analyses. Graphs of individual models were plotted with prediction bounds. 95% confidence levels were used to statistical evaluation of goodness of fit of models and prediction bounds.

Richards model was evaluted as the most corresponding with experimental data. Similar results were reached using Verhulst model. Von Bertalanffy model was not coresponded with experimental data.

growth models, bacterial growth, *Thermus aquaticus*, *Thermus scotoductus*

The first discovery of representatives of the genus *Thermus* have been a stimulus for a detailed study of physiology of thermophilic bacteria. Its discovery was very simple, because the cultivation is very easy. It was used salt synthetic medium that proved suitable for the growth of algae found in hot springs (BROCK, 1978). Representatives of the genus *Thermus* are Gram-negative aerobic bacteria, however some newly isolated species grow in anaerobic environments where nitrates used as the final electron acceptors. This genus does not form endospores (BALKWILL *et al.*, 2004). The morphology of the organism is largely influenced by temperature and growth phase. The organism is usually present in fibers when the temperature is above 75 °C. The fibers are formed only in the stationary growth phase culture at 65 to 70 °C (BROCK, 1978).

Members of the genus *Thermus* family are used to produce thermophilic compounds, especially enzymes, such as *Taq* polymerase (DNA polymerase), serine proteases, glucosidases, pullulanase (ČEJKOVÁ, 2009). Because are these products mostly secondary metabolites, it is necessary to know the growth characteristics of microorganisms that can also be described by mathematical models. *Taq* is frequently used in polymerase chain reaction (PCR).

Mathematical models are used to describe the behavior of microorganisms under different physical or chemical conditions such as temperature, pH and water activity. In order to build these models, growth has to be measured and modeled. Microbial growth often shows a lag phase in which the specific growth rate starts at a value of zero and then increases. The specific growth rate reaches to a maximal value in exponential phase. Then it is decreased of growth rate value and reached to zero in stationary phase. Investigators need adequate models to describe a growth curve and to reduce measured data. A number of models are found in the literature, such as the models of Verhulst, von Bertalanffy, Richards, and others. These models describe only the number of organisms and do not include the consumption of substrate as a model based on the Monod equation would do (ZWIETERING *et al.*, 1990).

The objective of this work is to evaluate similarities and differences between the models and experimental data. It deals with the question of which model(s) can be used, on the basis of statistical evaluation.

MATERIALS AND METHODS

Microorganisms and medium

Thermus aquaticus CCM 3486 and *Thermus scotoductus* CCM 4465, obtained from Czech collection of microorganisms (Brno, Czech Republic), was used throughout the study. All growth experiments were conducted in medium with composition: yeast extract 4 g, polypeptone 8 g, NaCl 2 g and distilled water 1 000 ml.

Cultivation conditions

Fermentation experiments were carried out using the bioreactor BioFlo/CelliGen 115. Parameters of cultivation were based on previously made by optimalization. Medium was regulated at pH = 6 for *T. aquaticus* and at pH = 7 for *T. scotoductus*. The temperature was maintained at 65 °C. Stirring was set at 150 rpm. Samples (10 ml) were taken at 1hour intervals.

Determination of growth curves

The optical density (OD) at 600 nm was measured using an Ultrospec cell density meter. The sample was centrifuged at 6 000 rpm for 6 min to determine the biomass concentration. The sediment was washed twice with distilled water. The washed sediment was quantitatively transferred to a dish and dried at 105 °C to constant weight. The concentration (c) was calculated as g of dry biomass by 1 liter of medium.

Mathematical modeling

Several sigmoidal function (Verhulst, Von Bertalanffy and Richards) were used to describe a bacterial growth curve. The modelling was performed using program MATLAB.

The Verhulst logistic equation is also referred in the literature as the Verhulst-Pearl equation after Verhulst, who first introduced the curve, and Pearl, who used the curve to approximate population growth in the United States in 1920 (TSOULARIS, WALLACE, 2002). Logistic equation was created by adding “saturation” in the exponential equation. “Saturation” can appear in limitation of sources or of space to cultivation of microorganisms (KLÁN, 1998). The new variable is found in logistic equation and it expresses carrying capacity:

$$\frac{dN}{dt} = r \times N \times \left(1 - \frac{N}{K}\right).$$

By integrating the equation we obtain a solution:

$$N(t) = \frac{K \times N_0}{(K - N_0) \times e^{-rt} + N_0},$$

where N_0 is the population size at time t = 0, K is carrying capacity, r is the intrinsic growth rate and represents growth rate per capita (TSOULARIS, WALLACE, 2002).

The von Bertalanffy model assumes that the growth of organisms is invariant on time (CLOERN, NICHOLS, 1978). Von Bertalanffy based his growth equation on model fish weight growth. This can be seen as a special case of the Bernoulli differential equation:

$$\frac{dN}{dt} = r \times N^{2/3} \times \left[1 - \left(\frac{N}{K}\right)^{1/3}\right].$$

By integrating the equation we obtain a solution:

$$N(t) = K \times \left[1 - \left[1 - \left(\frac{N_0}{K}\right)^{1/3}\right] \times e^{-\left(r \times t / 3 \times K^{1/3}\right)}\right]^3,$$

where N_0 is the population size at time t = 0, K is carrying capacity, r is the intrinsic growth rate and represents growth rate per capita (TSOULARIS, WALLACE, 2002).

The Richards growth model is found in literature as theta-logistic model or generalized logistic model too (LOIBEL, do VAL, ANDRADE, 2006). The theta logistic model is a generalization of the standard logistic equation. The theta logistic is defined as

$$\frac{dN}{dt} = r \times N \times \left[1 - \left(\frac{N}{K}\right)^{\beta}\right].$$

This equation is a special case of the Bernoulli differential equation too (TSOULARIS, WALLACE, 2002). Parameter β has not direct biological explanations, such as K, but allows greater variability in the shape of the curve (LOIBEL, do VAL, ANDRADE, 2006). By integrating the equation we obtain a solution:

$$N(t) = \frac{N_0 \times K}{\left[N_0^{\beta} + \left(K^{\beta} - N_0^{\beta}\right) \times e^{-\beta \times r \times t}\right]^{1/\beta}},$$

where N_0 is the population size at time t = 0, K is carrying capacity, r is the intrinsic growth rate and represents growth rate per capita (TSOULARIS, WALLACE, 2002) and β is the population intrinsic factor (LOIBEL, do VAL, ANDRADE, 2006).

RESULTS AND DISCUSSION

The mathematical models (Verhulst, Von Bertalanffy and Richards) were fitted to experimental data. The fitting was made in program MATLAB and from it were obtained the coefficients of models, the graphical display of individual models and the statistical evaluation of goodness of fit.

Application of the mathematical models to fit microbial growth curves

Fig. 1–12 shows the fitting of models to experimental data of growth curves. Fig. 1–3 shows growth *Thermus aquaticus* with measuring concentration.

Fig. 4–6 shows growth *Thermus aquaticus* with measuring optical density.

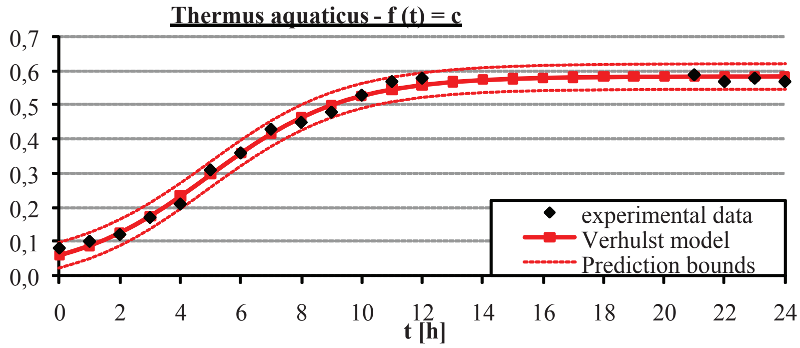
Fig. 7–9 shows growth *Thermus scotoductus* with measuring concentration.

Fig. 10–12 shows growth *Thermus scotoductus* with measuring optical density.

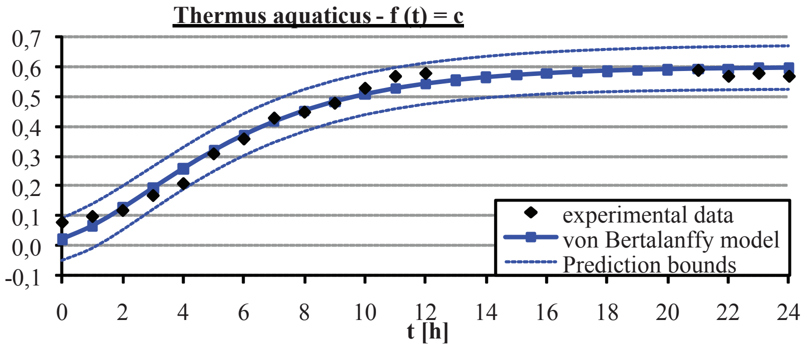
Prediction intervals were calculated and graphically displayed for each mathematical model of 95% confidence levels. These intervals can predict the behavior of culture in the future.

The coefficients of these models are displayed in Tab. I.

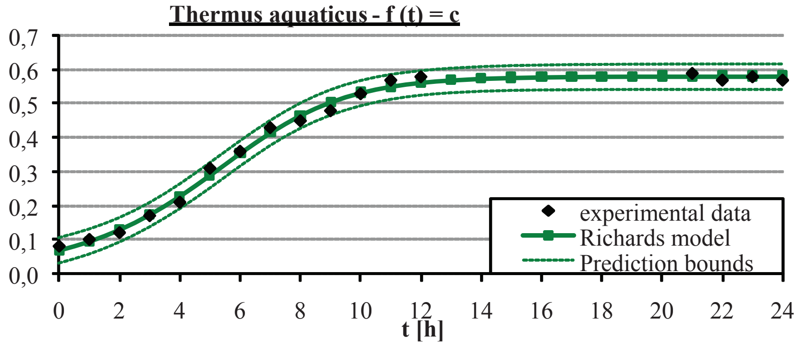
Their values determine the form of curves. They were found out after fitting experimental data to mathematical model. The coefficient *K* is carrying capacity, N_0 is the population size at time t = 0, *r* is the intrinsic growth rate and represents growth rate



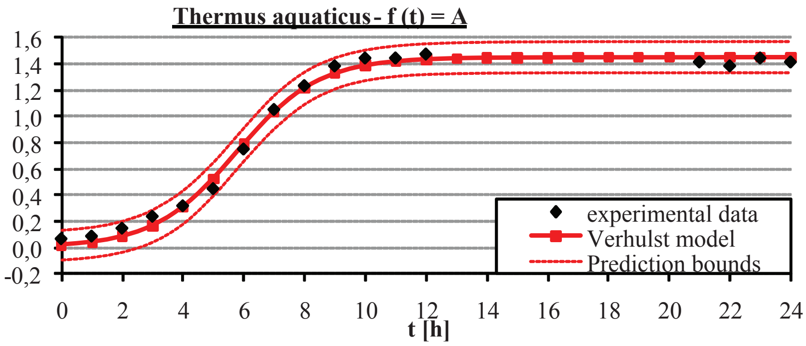
1: *Verhuls model fitted to data of Thermus aquaticus with measuring biomass concentration*



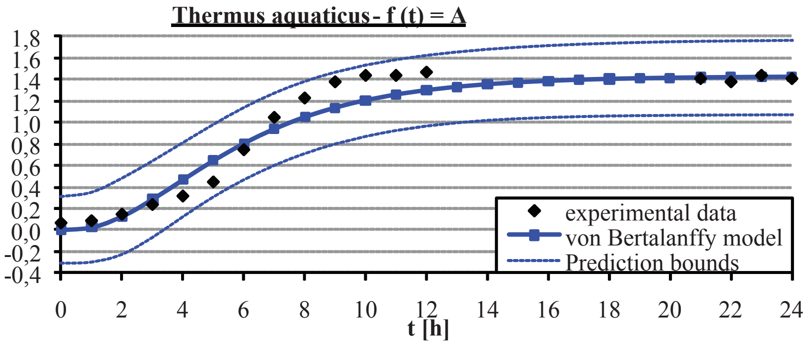
2: *Von Bertalanffy model fitted to data of Thermus aquaticus with measuring biomass concentration*



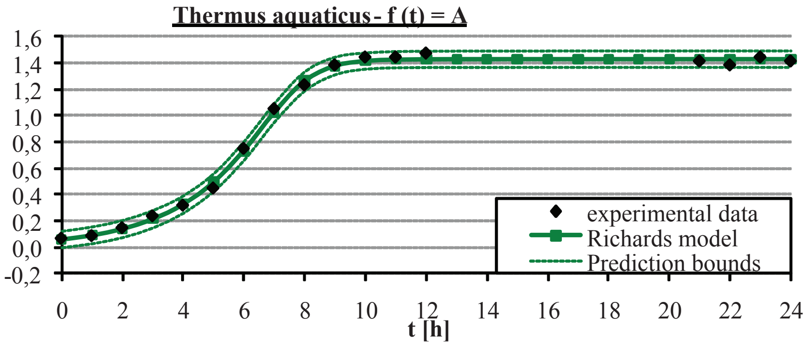
3: *Richards model fitted to data of Thermus aquaticus with measuring biomass concentration*



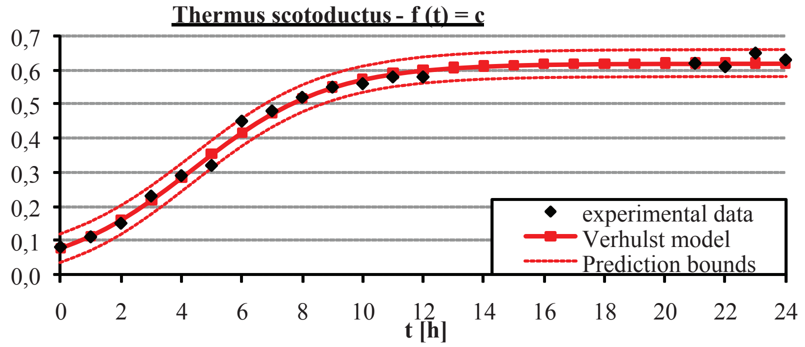
4: *Verhuls model fitted to data of Thermus aquaticus with measuring optical density*



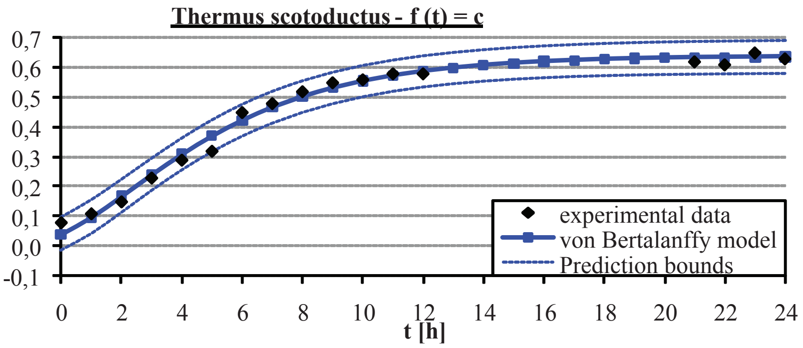
5: *Von Bertalanffy model fitted to data of Thermus aquaticus with measuring optical density*



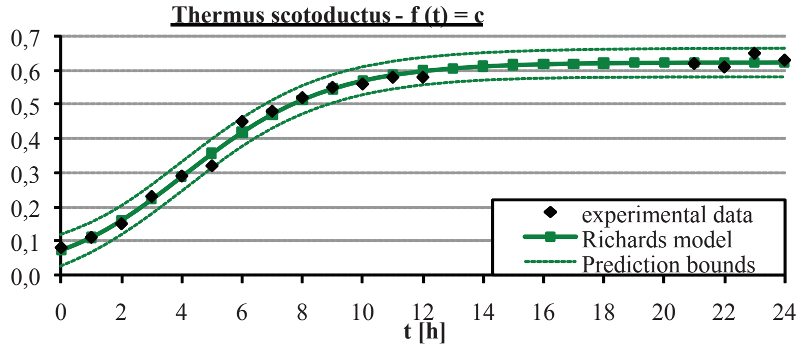
6: *Richards model fitted to data of Thermus aquaticus with measuring optical density*



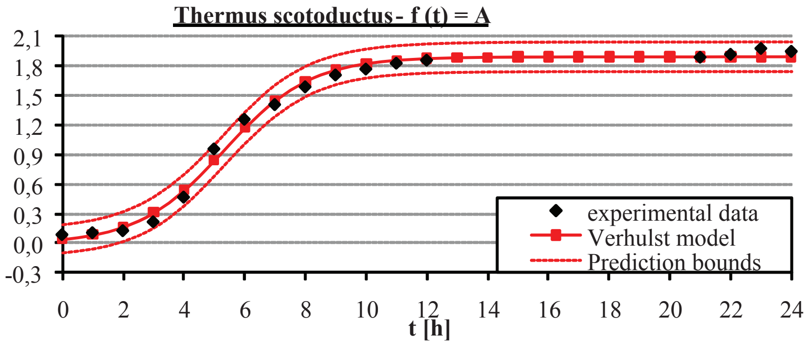
7: *Verhuls model fitted to data of Thermus scotoductus with measuring biomass concentration*



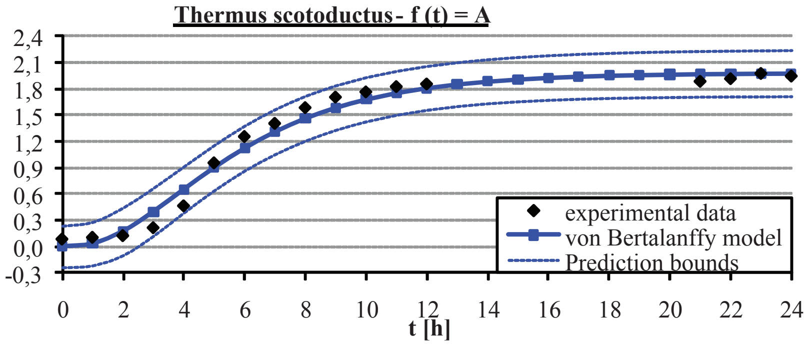
8: *Von Bertalanffy model fitted to data of Thermus scotoductus with measuring biomass concentration*



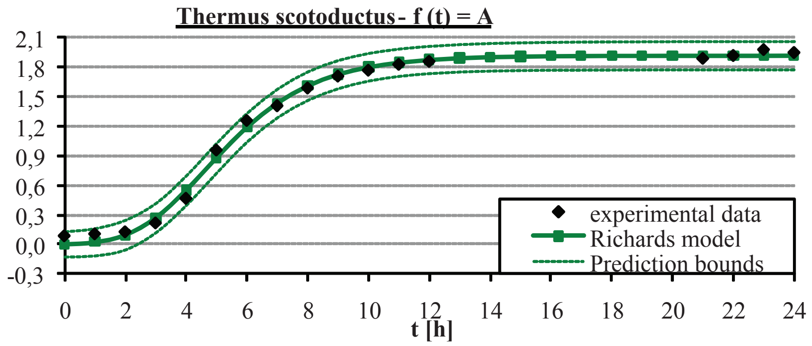
9: *Richards model fitted to data of Thermus scotoductus with measuring biomass concentration*



10: *Verhuls model fitted to data of Thermus scotoductus with measuring optical density*



11: *Von Bertalanffy model fitted to data of Thermus scotoductus with measuring optical density*



12: *Richards model fitted to data of Thermus scotoductus with measuring optical density*

I: *The coefficients of models*

	Verhulst			von Bertalanffy		
	K	**N_0**	**r**	**K**	**N_0**	**r**
Thermus aquaticus f(t) = c	0.5841	0.0599	0.4397	0.5979	0.0214	0.6387
Thermus aquaticus f(t) = OD	1.4480	0.0205	0.7372	1.4260	$5.06\cdot10^{-7}$	0.9809
Thermus scotoductus f(t) = c	0.6209	0.0782	0.4429	0.6376	0.0399	0.6650
Thermus scotoductus f(t) = OD	1.8940	0.0448	0.7012	1.9750	$3.25\cdot10^{-7}$	1.1020

	Richards			
	K	**N_0**	**r**	**b**
Thermus aquaticus f(t) = c	0.5809	0.0694	0.3348	1.5520
Thermus aquaticus f(t) = OD	1.4280	0.0607	0.4213	3.2760
Thermus scotoductus f(t) = c	0.6237	0.0720	0.5691	0.7024
Thermus scotoductus f(t) = OD	1.9180	0.0048	2.7490	0.1882

Note:
N_0 – population size at time t = 0,
K – carrying capacity,
r – the intrinsic growth rate and represents growth rate per capita,
β – population intrinsic factor.

per capita and β by Richards model is the population intrinsic factor. The different values of parameters N_0 and *r* are given by another form of equations of models. The parameter *K* is similar by all models, because it determines the maximum population size which should be the same.

In cases Verhulst model a Richards model, a good agreement between experimental data and predicted values was obtained. The results of the model according to von Bertalanffy equation are less consistent. Only one is outside the 95% confidence levels. This fact is also confirmed by statistical evaluation (of 95% confidence levels) of goodness of fit of models (Tab. II). The value R-square was around 99% in the Verhulst and Richards model. These results confirm the graphical display, where you can see a better fit of experimental data to curve model than by von Bertalanffy model.

The differences are probably due to different measurement procedure and the various equations of models. All results in this study are useful for predicting the behavior of microorganisms during the cultivation under the same conditions.

CONCLUSIONS

Growth curve data collected during the cultivation was used to determine the suitability of selected models. The results show that the Richards model and the Verhulst model have the goodness of fit of models (R-square) around 99%. From this it can be concluded that the equations of these models are suitable for further modeling. Von Bertalanffy model has achieved R-square below 95% once. It is therefore possible to say that this model is useful only at a lower confidence levels.

II: *The statistical evaluation of goodness of fit of models*

	Verhulst			von Bertalanffy			Richards		
	SSE	**R-square**	**RMSE**	**SSE**	**R-square**	**RMSE**	**SSE**	**R-square**	**RMSE**
Thermus aquaticus f(t) = c	0.0036	0.9939	0.0160	0.0132	0.9774	0.0307	0.0031	0.9946	0.0155
Thermus aquaticus f(t) = OD	0.0371	0.9929	0.0515	0.2975	0.9429	0.1458	0.0107	0.9979	0.0287
Thermus scotoductus f(t) = c	0.0042	0.9932	0.0174	0.0078	0.9875	0.0235	0.0041	0.9934	0.0177
Thermus scotoductus f(t) = OD	0.0616	0.9931	0.0663	0.1734	0.9804	0.1113	0.0443	0.9950	0.0584

Note:
SSE – sum of squares due to error,
RMSE – root mean squared error.

SUMMARY

The aim of this study was to compare the suitability of selected mathematical models fitting to experimental data. To test were selected following models: Verhulst model, the von Bertalanffy model and the Richards model. These models do not represent the substrate consumption but only the number of cells. Their equations are derived from the exponential growth equation. Experimental data were obtained by cultivation of *Thermus aquaticus* and *Thermus scotoductus*. The cultivation was performed in a medium composed of: yeast extract 4 g, polypeptone 8 g, NaCl 2 g and distilled water 1 000 ml. The number of cells was determined as optical density and as concentration. Optical density was measured using the Ultrospec cell density meter. The sample (10 ml) was centrifuged. Washed sediment was dried. The concentration of cells was calculated from dry weight. The program MATLAB was used for mathematical modeling. With it were evaluated parameters of individual models, plotted graphs with prediction intervals and forecasting statistically evaluated goodness of fit of models. The graphical display and statistical evaluation shows that the Verhulst model and the Richards model achieve similar results, which are very accurate. The results of the von Bertalanffy model show less goodness. One result is outside the 95% confidence levels. Others R-square values ranging over the value of 97%. All results in this study are useful for predicting the behavior of microorganisms during the cultivation under the same conditions. From the results it can be concluded that for curves fitting to experimental data are preferable to models by Verhulst and Richards than the von Bertalanffy model.

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