Article

On the relationship between the observed dynamics of a colorimetric indicator and the nonlinear dynamics of the population growth under study in the case of microbial cultures

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Abstract. The resazurin test is one of the most widespread approaches for studying the growth and metabolic activity of microorganisms. It is based on the colour change of the blue indicator, resazurin, to its pink reduced form, resorufin due to the reduction process catalyzed by the metabolic activity. At the same time, the quantitative characterization of the process needs to take into account the fact that one registers the results of the chemical transformation, which can differ from the underlying kinetics of the population growth. *Purpose.* The principal goal of this work is a sequential modelling of both coupled nonlinear growth processes aimed at obtaining the closed-form solution depending on the specificity and parameters of biological and chemical counterparts and its comparison with the curves obtained experimentally. *Methods.* The indicator concentration change is derived under the assumption of the logistic bacterial growth catalyzing the unidirectional chemical reaction considered and compared with the photometrically registered growth curve for a population of lactobacteria. *Results.* It is revealed that the biochemical growth curve will be logistic too only in the case of specially coordinated kinetic parameters and the systems' carrying capacity. Otherwise, another functional form should be used to approximate the observable dynamics. *Conclusion.* Thus, the main conclusion consists of drawing attention to the importance of distinguishing between the underlying microbial and observable chemical growth curves. Their difference affects the value of the population growth rate, which is the target of such tests, and, therefore, the proper functional form should be used for the experimental data regression.

Keywords: resazurin, resorufin, cellular activity, population dynamics.

Introduction

Colorimetric and spectrophotometric methods based on recording the change in color (or fluorescence) of an indicator added to a liquid medium with microorganisms cultivated in it currently occupy a place among the main methods for characterizing population growth in

fundamental and applied microbiology [1, 2]. This is primarily due to the fact that such an approach is significantly simpler than visual microscopic counting of individual microorganisms or colony-forming units (CFU), and is more reliable than recording the turbidity of the medium by its total optical density (OD), since the latter approach does not distinguish between actively dividing, viable and non-viable organisms. In contrast, specially selected indicators respond to enzyme-substrate reactions within a living cell, respiratory activity, and/or other types of metabolic activity, allowing one to solve the required problems of determining the population dynamics of microorganisms [3]. Today, these methods are of particular interest in the context of developing remote monitoring systems [4], which enable automated recording and transmission of data on bacterial contamination, screening of responses to antibacterial agents, etc.

However, it should be noted that there is a fundamental difference between a certain growth of a microorganism population, the model description of which is based on well-developed nonlinear differential equations (Verhulst, Gompertz, and others), verified using direct CFU counting methods [5–7], and the observed dynamics, which are caused by both biochemical reactions and the relationship between the quantitative parameters of the selected color scale and the concentrations of the indicator substance forms.

Known methods establishing such a correspondence mainly operate with empirical dependencies, usually based on the selection of reagent concentrations in such a way that a linear correlation is observed between the microscopic count of CFU or the determination of OD and the photometric curve when filtering the image in a selected narrow range of wavelengths approximately correspon-ding to the color of interest of the indicator [8]. The correspondence of the change in the color of the indicator to the detection threshold time of the presence of microorganisms starting to grow and reproduce with different initial concentrations [9], the change in the magnitude of the main components in a given color space [10] or the correlation established by machine learning [11, 12] can also be used. The number of works devoted to the corresponding analysis based on mathematical models operating with coupled dynamic systems is very limited.

A simple kinetic model combining linear kinetic equations with coupled parameters for population growth and optical density change was considered in [13]. A much more complex system, taking into account the reduction of the nutrient medium revealed by colorimetric analysis and the corresponding growth of biomass based on empirically selected equations and parameters, was used in a numerical model of a bioreactor in [14]. In [15], it was shown experimentally that different initial dilutions of the mycobacterial culture can lead to a change in the type of differential equation describing the dynamics of the fluorescence of the indicator medium during population growth, from the Verhulst model to the Gompertz model, and a qualitative explanation was proposed due to different ratios of the kinetic constants of population dynamics and photochemical kinetics. A number of kinetic models linking the detectable color response of the resazurin indicator with biochemical kinetic processes are presented in the review [16].

Thus, the main goal of this work is to build a sequence of models from the population growth of a bacterial culture to the recorded dynamics of the color of the indicator, which is resazurin (7-hydroxy-3H-phenoxazin-3-one-10-oxide, also known under the commercial name Alamar Blue), which, when interacting with mitochondrial and cytoplasmic reductases of a living cell, is reduced to pink resorufin (7-hydroxy-3H-phenoxazin-3-one) with further possible transformation into colorless dihydroresorufin (7-hydroxy-1,2-dihydro-3H-phenoxazin-3-one). This indicator is one of the main reagents in modern microbiological studies of the viability of cell cultures [2, 17]. The chemical kinetics of its reduction reaction is well studied [18, 19]. In addition, for this reaction, the correspondence of concentration dependences obtained by spectral analysis to colorimetric and photometric characteristics has now been established [20]. The use

of lactobacilli as a model culture is based on a detailed study of the corresponding population growth dynamics using a wide range of classical nonlinear differential equations based on the CEE calculation method [6].

1. Model of coupled population and chemical kinetics

The kinetics of the resazurin reduction reaction catalyzed by reagent R proceeds according to the scheme [18, 19]

$$\text{X} \xrightarrow{\text{kR}} \text{Y} \underset{k'^{-1}_- R}{\overset{k'_+ R}{\rightleftharpoons}} \text{Z}, \tag{1}$$

where X is resazurin, Y is resorufin, Z is dihydroresorufin, which is a non-fluorescent transparent substance. This second reversible step is usually not used for the viability test of microorganisms, so if we neglect the conversion of resorufin to dihydroresorufin and consider only the first-order reaction catalyzed by the number of microorganisms $N(t)$ with the proportionality coefficient (the rate of the irreversible chemical reaction) k,

$$\text{X} \xrightarrow{\text{kN(t)}} \text{Y},$$
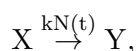
then for the concentrations (x and y, respectively) we have

$$\frac{dx}{dt} = -kN(t)x, \tag{2}$$

$$\frac{dy}{dt} = kN(t)x, \tag{3}$$

with the initial conditions $x(0) = x_0$, $y(0) = 0$ and the conservation law $x + y = x_0$, where x_0 is the initial concentration of resazurin; the initial concentration of resorufin in this case is zero.

From integration (2) by the method of separation of variables it follows that

$$\ln\left(\frac{x}{x_0}\right) = -k \int\limits_0^t N(t)dt. \tag{4}$$

Thus, the sought solution (the kinetic curve of the indicator concentration growth) can be a non-trivial function of time depending on the time evolution of the microorganism population and has the form

$$y(t) = x_0 \left(1 - e^{-k \int\limits_0^t N(t)dt}\right). \tag{5}$$

Note that the solution (5) depends on time even in the case when the population is constant in number but viable, i.e. $N = N_s = \text{const}$, and the concentration of the registered resorufin grows, exponentially approaching the stationary state as

$$y(t) = x_0 \left(1 - e^{-kN_s t}\right). \tag{6}$$

Thus, the trivial fact of the presence of a time dependence of the indicator concentration change on time does not indicate the presence of growth of the microbiological culture and speaks only of its viability. At the same time, an observation of importance for practical pharmacological applications [21] is known about the difference between the minimum inhibitory concentration

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of a drug (MIC) (the concentration leading to the death of pathogenic microorganisms) and the steady-state concentration, at which the growth rates of the death of organisms are compared, which leads to the emergence of a stationary but viable population. The time dependence reflected in the solution (6) indicates that both cases (the steady-state concentration of the drug and the concentration significantly below the MIC) lead to a change in the indicator readings and a more accurate conclusion should be based not on the fact of their growth, but on a more accurate analysis of the corresponding functional dependence (see below). A specific variant of such behavior was also noted in the work [15], where the curve of growth of tuberculosis mycobacteria in a certain time interval was composed of a sequence of sections of curves of the type (6), and only their initial and final points fit the classical Verhulst microorganism growth curve. Such behavior led to the interpretation of the growth of the mycobacterial culture as synchronized moments of divisions, between which the number of colonies does not change, as follows from (6).

The second factor that must be taken into account: the exit of the growth curve of the indicator substance to the steady state does not necessarily mean the exit of the growth of the microbiological culture to the steady state, since this can occur simply due to the exhaustion of the amount of the indicator. In particular, this can be clearly demonstrated by substituting into the equation (5) the function of unlimited population growth $N(t) = N_0 \exp(rt)$ with the initial value N_0 and the reproduction constant r, which gives an exceptionally fast (double exponential) growth curve of the indicator that reaches a stationary state

$$y(t) = x_0 \left(1 - e^{-kN_0\left[e^{rt}-1\right]}\right). \tag{7}$$

Therefore, let us now consider in detail a more realistic case of growth in the number of microorganisms, which satisfies the classical equation of logistic growth — the Verhulst equation

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) \tag{8}$$

with the initial population $N(0) = N_0$, the population growth constant r, the capacity of the environment K, and has a solution

$$N(t) = \frac{N_0 e^{rt}}{\frac{N_0}{K}\left(e^{rt} - 1\right) + 1} \equiv \frac{K e^{rt}}{e^{rt} + (K/N_0 - 1)}. \tag{9}$$

Substituting the logistic function (9) into the solution for the substrate concentration (4), we arrive at the integral

$$\int_0^t N(t)dt = \int_0^t \frac{N_0 e^{rt}}{\frac{N_0}{K}\left(e^{rt} - 1\right) + 1}dt,$$

which is taken in analytical form, and we obtain

$$\ln\left(\frac{x}{x_0}\right) = -\frac{kK}{r}\ln\left[\frac{e^{rt} + (K/N_0 - 1)}{K/N_0}\right].$$

As a result, the solutions for the time evolution of the concentrations of resazurin and resorufin have the form

$$x = x_0 \left[\frac{K/N_0}{e^{rt} + (K/N_0 - 1)}\right]^{\frac{kK}{r}} \tag{10}$$

and

$$y = x_0 \left(1 - \left[\frac{K/N_0}{e^{rt} + (K/N_0 - 1)} \right]^{\frac{kK}{r}} \right) \tag{11}$$

respectively.

Clearly, none of these are logistic functions, although the expression (10) can be viewed as a solution to the nonlinear Richards equation [22] in reverse time,

$$\frac{dx}{dt} = -x_0 kK \left(\frac{x}{x_0} \right) \left(1 - \left(\frac{x}{x_0} \right)^{\frac{r}{kK}} \left[1 - \frac{N_0}{K} \right] \right), \tag{12}$$

also known as the generalized logistic growth equation.

The factor in square brackets can be expressed in terms of x from (10):

$$\frac{dx}{dt} = -x_0 kN_0 \left(\frac{x}{x_0} \right)^{\frac{\frac{kK}{r}+1}{\frac{kK}{r}}} e^{rt} \equiv -x_0 kN_0 \left(\frac{x}{x_0} \right)^{\frac{r}{kK}+1} e^{rt}.$$

The remaining exponential factor can again be expressed from (10), and, by virtue of the conservation law $x + y = x_0$, the solution (11), corresponding to the curve of growth of resorufin concentration, satisfies the equation

$$\frac{dy}{dt} = x_0 kK \left(1 - \frac{y}{x_0} \right) \left(1 - \left(1 - \frac{y}{x_0} \right)^{\frac{r}{kK}} \left[1 - \frac{N_0}{K} \right] \right). \tag{13}$$

However, the curve of growth of indicator concentration (11) admits a special case for the combination of parameters $kK/r = 1$, in which the function under consideration takes the form

$$y_V = x_0 \frac{[e^{rt} - 1]}{[e^{rt} - 1] + [(K/N_0 + 1) - 1]}. \tag{14}$$

Comparison of formulas (9) and (14) shows that they have a similar fractional rational structure with the exponential function of time in (9) replaced by a shifted exponential function in (14), and the ratios of the final and initial population sizes shifted by the same unit. The first shift naturally follows from the fact that at the initial moment, resorufin, unlike microorganisms, is not present in the system, and the solution for its concentration must be shifted to zero. The shift of the asymptotic stationary state is essentially a consequence of the same difference. This conclusion about the reduction of the generalized logistic growth to the standard one can be reached by directly substituting $kK/r = 1$ into the equation (13), which actually takes the form of the Verhulst equation for the variable $1 - y$, written for inverse time (due to the symmetry of the logistic curve with respect to the inflection point and the asymptotics in time, the formal direction of time does not affect the characteristic shape of the logistic curve).

Since the exponential function is growing quite rapidly, then for times significantly exceeding the inverse value of the characteristic growth constant of microorganisms ($t \gg r^{-1}$), the subtracted unit can be neglected, that is, we obtain the logistic Verhulst function

$$\tilde{y}_V = x_0 \frac{e^{rt}}{e^{rt} + [(K/N_0 + 1) - 1]}, \tag{15}$$

growing with the same reproduction constant as the studied population, but reaching the saturation value corresponding to the maximum concentration of the indicator, resorufin, at its complete

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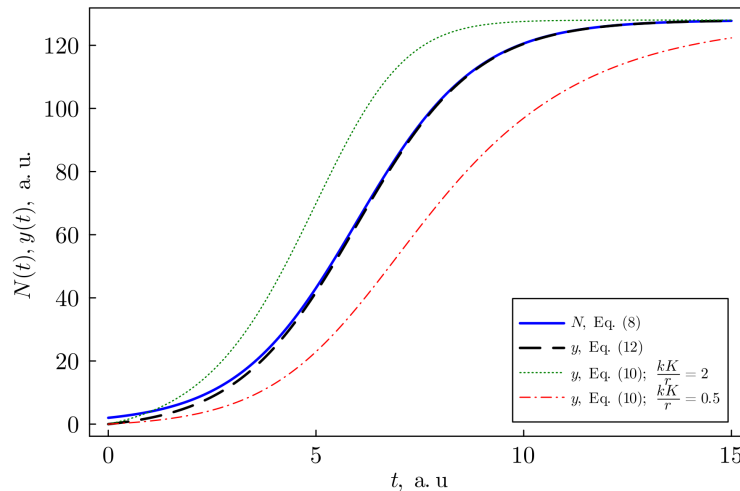
Fig 1. An illustration comparing the kinetic curves for population growth and the growth of the recorded resazurin concentration at different values of the control parameter kK/r. Initial conditions are $N_0 = 2$, $y(0) = 0$; the asymptotic stationary values are matched in magnitude for clarity: $K = x_0 = 128$, the growth constant $r = \ln(2)$ is chosen in such a way that the characteristic time of population doubling corresponds to the time unit $T_d = 1$

conversion from resazurin. Given the relationship between the growth constant r and the population doubling time $T_d = \ln(2)r^{-1}$, it is clear that regression using the logistic growth function is applicable at least after several characteristic division times.

Fig. 1 illustrates these findings, showing that in the special case of $kK/r = 1$ the curve of resorufin concentration growth is virtually indistinguishable from the population growth curve after about five cell division cycles and can be adequately used to characterize the process (in principle, given the relatively small discrepancy between the curves and the fact that real experimental data have a certain error, a logistic curve can be used for their regression even after about three division cycles). In practice, this correspondence between the resazurin conversion curve and the population curve determined by direct counting of bacterial colonies was found for the resazurin test for standard cell cultures [23] and in comparison with alternative methods for monitoring the metabolic activity of viable strains of *M. tuberculosis* [24]. However, it is noted that this observation requires certain conditions for the concentrations of the culture and reagents, which confirms the conclusion made about the need to match the parameters.

At $kK/r > 1$, the rapid conversion of resazurin to resorufin leads to the recorded curve reaching saturation much earlier than the stabilization of population growth occurs, since there is actually no reagent left in the system. On the contrary, at $kK/r < 1$, the above-mentioned effect is observed (see equation (6)) that the stabilized viable population continues to perform respiratory activity, which leads to the restoration of resazurin still remaining in the system to resorufin, the concentration of which increases over time.

2. Analysis of experimental data based on the population-chemical model

2.1. Materials and methods of the experiment. The microbiological objects chosen were the microbial mass of a live antagonistically active strain of lactobacilli (*Lactobacillus plantarum 8P-A3* or *Lactobacillus fermentum 90T-C4*) with the addition of components of the protective drying medium (gelatin, sucrose, milk) (AO NPO Mikrogen, Moscow, Russia). These components also provide the growing culture with nutrients when diluting the suspension with

water. As a result, no additional nutrient medium was used. Dry lactobacilli (CFU not less than $1 \cdot 10^{10}$ in 15 g of lyophilisate) were diluted in 250 ml of distilled water, after which they were kept with continuous stirring for 30 minutes to ensure uniform distribution of microorganisms throughout the volume. A series of 11 lactobacillus suspension samples was then prepared, each with a volume of 10 ml from the initial concentration to a dilution of 11 times. To each of the solutions, 1 ml of a diluted resazurin solution was added. As a control, 10 ml of boiled distilled water with an equivalent amount of dye was used.

Preparation of the indicator stock solution (sodium resazurin): distilled water was boiled for 5 minutes and then cooled to $(25 \pm 2)°C$, after which (0.055 ± 0.001) g of sodium resazurin (Sigma-Aldrich (Burlington, MA, USA), dye content more than 75%) were added. The mixture was thoroughly mixed until the dye was completely dissolved. The resazurin solution was diluted $1 : 3$ with distilled water. The concentration of resazurin in the working solution was 0.00067 mol/l.

A portable microbiological analyzer (PMA) [25] (Patent RU 2 779 840 C1) was used to obtain photometric curves for 72 hours with a data recording frequency of — every 15 min. The analysis presented in [20] indicates the existence of a linear relationship between the concentration of resorufin and the recorded illumination of the photocells in the mode before the transition to the stage of conversion of resorufin to dihydroresorufin. Thus, the instrument readings reflect the change in the amount of the product of the chemical reaction y.

2.2. Results and discussion. Fig. 2, a shows an example of the indicator color change in a series of wells of a microbiological plate for one time point after the start of measurements under the condition of different initial concentrations of lactobacilli. It is evident that this range of initial dilutions over 72 hours of the process leads to a full version of colorimetric reactions (1): from a virtually unchanged blue control color through pinking to a completely transparent solution indicating complete conversion to dihydroresorufin. In this regard, we note that within the framework of the model limited by the irreversible conversion of resazurin to resorufin, the variants of the kinetics of rapid decolorization (dilutions greater than $(1/7)$) are not analyzed in detail.

In Fig. 2, b for each of the cells of the plate, the green and crimson markers display the PMA readings normalized to the value of illumination at the initial moment of time to correct for its variability over the area of the plate. Due to the applied normalization $y(0)/y0 = 1$, while the absence of resorufin in the system represents its zero concentration, the data fitting by the solution of the Verhulst equation should be carried out with a shift by a constant d, that is,

$$\frac{y}{y_0} = \frac{K - d}{1 + e^{-r'(t - t_m)}} + d, \tag{16}$$

where t_m is the time moment corresponding to half the growth.

Note that the logistic growth model (16) by definition requires a non-zero (for $y/y_0 - d$) initial value, since the Verhulst kinetics is autocatalytic. In addition, it is evident from Fig. 2, b that for a number of cells of the plate a certain drop in illumination is detected during the first hours after the start of the experiment, associated with the evaporation of the liquid condensing on the upper cover of the plate, which reduces the transparency of the latter. As a result, when performing a nonlinear regression with the objective function (16), the parameter d should also be determined in order to take into account the effect of normalization taking into account the condensate. Nonlinear regression using the function (11) is also performed with an additive introduction of a curve shift by a constant value.

In addition, it is evident that, starting from the dilution $(1/10)$, the nature of the signal growth undergoes changes, which is associated with the second step of the reaction (1). Therefore,
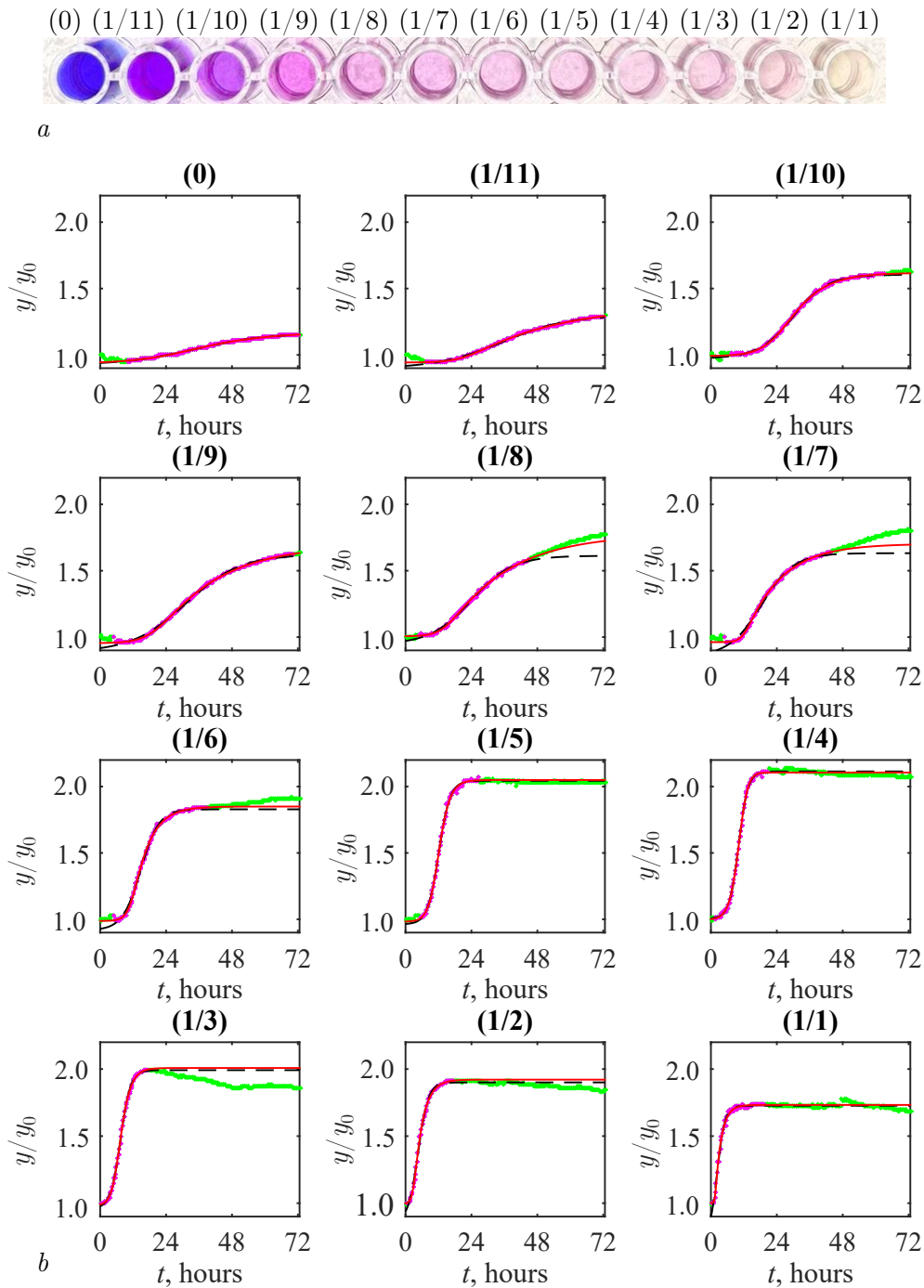
Fig 2. *a* — A photo of microbiology plate wells taken 72 h after the start of the experiment; the relative initial dilutions in fractions of $4 \cdot 10^8$ COU are designated above each well; designation (0) corresponds to the resazurin control without culture addition. *b* — Photometric data obtained with PMA normalized to the initial illumination (magenta (used for the subsequent fitting) and green markers); data fitted by a logistic curve (black dashed line) and by solving the population-chemistry model equation (solid red line) (color online)

only the part of the data corresponding to the active transient process was used for the subsequent regression (the corresponding markers are highlighted in crimson).

From the obtained experimental data (see Fig. 2, *b* for subpanel (0)) it can be noted that the control solution shows a slight increase. This can be explained by the mass transfer of lactobacilli metabolites or lyophilizing substances of the solution, some of which could get into the well with pure resazurin through the condensate formed on the surface of the plate lid. Their trace amounts can lead to the resazurin reduction reaction, especially under the condition of exposure to light regularly illuminating the plate during the recording of photometric data [26, 27]. However, this increase in resorufin concentration is quite insignificant, and even after a long time from the start of the experiment, the colorimetric characteristics of the solution do not change significantly, and it remains blue. This interpretation is supported by the observation of the response dynamics of the most diluted lactobacilli solution (1/11), which shows almost equivalent growth until the moment when the bacteria enter the active growth phase (after about 65 hours) and their number is sufficient to increase the noticeable dynamics of vital activity. This comparison will be discussed below.

Dilutions (1/10) and (1/9) show an almost perfect superposition of the experimental values on the chemical kinetics curve of resazurin reduction and the growth curve corresponding to the solution of the Verhulst equation within the resolution of the graph, and reaching a steady-state value by the end of the experiment. The transition of resazurin to resorufin in these cases is clearly visible by the saturated pink color in the photographs of the corresponding cells (see Fig. 2, *a*) at the moment the curves reach saturation (see Fig. 2, *b*).

On the other hand, (1/8), (1/7) and (1/6) lie on the logistic curve only up to a certain point. The latter (as well as the function (11)) demonstrates saturation, while the photometric data show the emergence of a new phase of faster growth. It corresponds to the rapid clarification of the medium in the cells due to the reaction of resorufin reduction to dihydroresorufin, which leads to significant dilution of the medium with a transparent product. This is also clearly visible in Fig. 2, *a*.

Finally, in the wells (1/5)–(1/1), the saturation of the photometric signal occurs so quickly that the two-stage process is not visible. In fact, with such amounts of catalysts (metabolites of lactobacilli), the reduction reactions proceed in parallel, and the solution quickly becomes clearer. Moreover, with the initial dilutions (1/4)–(1/2), a decrease in the photometric signal is visible instead of its stationarity. This is due to the turbidity of the medium and a decrease in the intensity of light transmission of the solution due to the appearance of a large number of lactobacilli, that is, with a direct change in optical density, no longer associated with chemical indicator processes (the medium remains transparent). Within the resolution of the graphs in Fig. 2, *b*, the curve (16) and the shifted curve (11) fit the data well, but it is clear that the saturation values are significantly (approximately one and a half times) higher than for the case of pinkened cells. However, these cases are beyond the scope of the model operating with only one irreversible reaction and will not be considered further.

It should be noted that the apparent resolution in the graphs in Fig. 2, *b* is quite rough and a detailed discussion of the issue of reproducing sigmoidal growth curves of photometric curves by solving model equations requires the presentation of data that are more sensitive to the type of kinetic curves. This analysis is presented in Fig. 3. It is based on the linearization of the curve given by the function (16) in the semilogarithmic representation

$$-\ln\left(\frac{K-d}{y/y_0-d}-1\right) = r't_m - r't, \tag{17}$$

where the shift of the resulting straight line $r't_m$ is determined by the initial conditions, and the

slope r' specifies the growth rate corresponding to the logistic equation.

Fig. 3 demonstrates the corresponding form of data and their approximations (on the regression interval) for those wells for which rapid bleaching due to the second stage of the reaction is not observed. In this representation, the significant approximation of the regression by the logistic function (16) becomes clearly noticeable: even in the central region of the best approximation, the markers corresponding to the experimental data are located in a wave-like manner around the fitting straight line. At the initial and final sections of the interval, the deviations of the markers' location from the straight line become quite significant. At the same time, the solid lines corresponding to the solution (11), taking into account both processes — population growth and the chemical reaction of the indicator — neatly pass along the essentially nonlinear curve of the experimental data location.

It should be noted that even a similar identical average slope of the approximating linear sections does not indicate the same rate of population growth determined by the two methods, although it leads to the same qualitative conclusions. First of all, attention should be paid to the curves (0) and (1/11), for which the slopes are, firstly, smaller than for the others, and secondly, they practically coincide for the control and the highest dilution. This indicates that the above assumption that the increase in the photometric signal for these cells is associated not with the reproduction of lactobacilli, but with possible side chemical reactions. An additional argument is that for the case (1/11) at the final moments of time ($t > 65$ h) a deviation from the straight line begins upward, associated with the excess of the insensitivity threshold by the number of lactobacilli and the corresponding effect of metabolic processes in viable cells on the conversion of resazurin. Further dependencies correspond to the situation when the indicator reaction is catalyzed by metabolic processes during the growth of the microorganism population, and not by accompanying substances in the solution, which is evident from the fact that the three dependencies — for the initial dilutions (1/10), (1/8), (1/7) — exhibit approximately the same characteristic growth rate, determined by the close slope of the straight sections in semi-logarithmic coordinates. The parallel shift between them corresponds to different initial conditions, which fully corresponds to the discussed kinetic models. The smaller slope for the
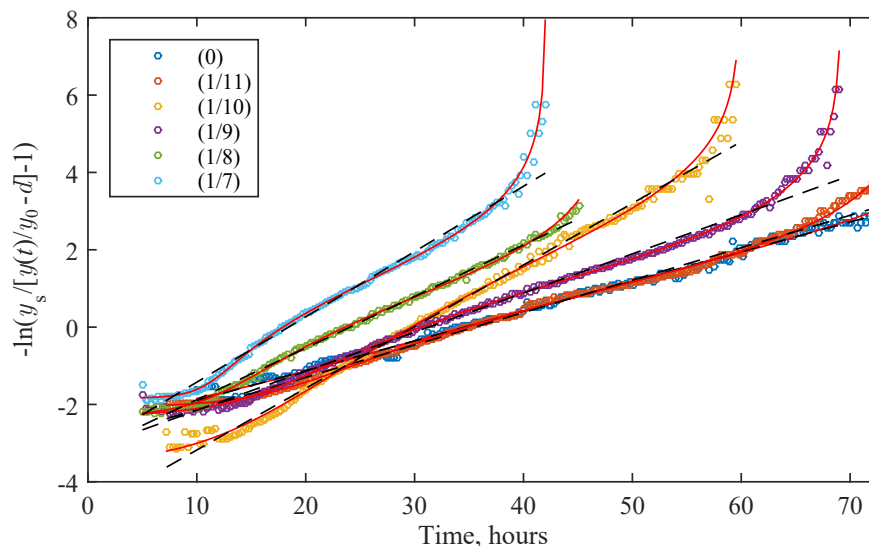

Fig 3. Experimental data (circles); chemical kinetics of resazurin (solid line); Verhulst equation (dashed line) (color online)

dilution (1/10) can be associated with the individual characteristics of that part of the culture that got into this cell (the corresponding graph in Fig. 2, b also shows the specificity of slower growth, which still does not lead to the beginning of the transition to the dihydroresorufin stage of the reaction in 72 hours).

However, the magnitude of the population growth rate itself is different according to the data of the Verhulst model and the more complete population-chemical model. The Verhulst model gives $r' = (0.15 \pm 0.018)$ h^{-1}, while the model (11) gives meanings $r_{(1/10)} = 0.21$ h^{-1}, $r_{(1/8)} = 0.33$ h^{-1}, $r_{(1/7)} = 0.67$ h^{-1} with power exponents $(kK/r)_{(1/10)} = 0.52$, $(kK/r)_{(1/8)} = 0.13$ and $(kK/r)_{(1/7)} = 0.11$. Thus, it can be seen that these indicators differ significantly from unity, that is, the model that takes into account the fact of registration of a chemical process, and not the reproduction of microorganisms directly, is not equivalent to the solution of the Verhulst equation, despite the similarity of the dynamics in the region of the inflection point of the recorded growth curve (but a significant difference at the stages of the beginning and end of the growth of the indicator curve of the conversion of resazurin to resorufin).

The difference in population growth rates for different initial dilutions may be due to a number of factors. One of them is the different concentration of the nutrient medium that was added together with the culture and diluted in the same proportion, i.e. the concentration per well volume of the plate varied, increasing from well (1/10) to (1/7). A more significant factor is the possible dependence on the initial concentration of lactobacilli due to the influence of the latter on the duration of the lag phase of growth dynamics. [28]. Modern research [29,30] argue for the existence of the need to take into account the quorum effect, the consequence of which is a lower characteristic growth rate of the culture due to the extension of the lag phase at low concentrations of colony-forming units, which corresponds to an increase in the constants from $r_{(1/10)}$ to $r_{(1/7)}$.

At the same time, the values of these constants correspond to the range of values found in a number of well-known works that addressed the direct counting of colony-forming units of lactobacilli or the optical density of their suspension, pre-calibrated to the values of direct counting. For the temperature range of 20...30°C, corresponding to our experiment, the growth parameter varies in the range of 0.30...0.65 h^{-1} [31,32] depending on the conditions of the growth environment, which corresponds to $r_{(1/8)}$ and $r_{(1/7)} = 0.67$ taking into account the experimental error. The value $r_{(1/10)}$ is below this range, but taking into account the above-mentioned effect of the decrease in the growth rate at large initial dilutions and the variability of growth in general, the discrepancy is not critical. At the same time, the use of the Verhulst model gives a significantly underestimated value, which testifies in favor of the relevance of the modified approach.

Thus, the development of more detailed models that take into account the diversity of population, biophysical and chemical processes opens up prospects for a clearer understanding of the entire complex of interrelated processes that determine the visible indicator response to the growth of a microorganism culture.

Conclusion

Thus, the main conclusion of this work is to draw attention to the need to clearly separate the observed kinetics of the measurement of the recorded signal and the true kinetics of population growth when using indicator media. Despite the fact that the obtained data can be approximated by classical sigmoid curves (such as the Verhulst, Gompertz, etc. dependences), at least some of the parameters of these curves, especially the characteristic growth constant, may not correspond

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to the real growth rate of microorganisms due to the fact that the process includes not only an increase in the concentration of the latter, but also directly the chemical reaction of reagent transformation. This is especially important for the case of fast-growing cultures, for which the rates of both processes are comparable. The result of this is the need to operate with a model that simultaneously includes the nonlinearity of the system and the non-stationarity of its parameters.

In this work, by means of consistent application of the described methodology, Gompertz (double exponential dependence) and Richards (generalized logistic growth) type solutions are obtained as kinetic curves corresponding to exponential and simple logistic growth of the population in the indicator medium. In this case, the dependence of the generalized logistic growth can be reduced to logistic growth over a sufficiently long time interval with an appropriate choice of the ratio between the kinetic constants of population reproduction, the chemical reaction of the indicator and the capacity of the medium. The possibility of such a match explains the known empirical direct proportionality between the concentration of resorufin and the number of colony-forming units in the resazurin test of mycobacterial cultures and substantiates recommendations for strict adherence to the conditions for conducting this test specific to various species and strains of mycobacteria. Deviation from the obtained dimensionless criterion leads to the need to use a more general functional dependence, which is demonstrated in a practical example of processing data on testing the growth of a fast-growing culture of lactobacilli.

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