Mathematical Modeling of Morphogenesis and Population Dynamics of Bacteria-Destructors during the Ellimination of Oil Pollution

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Abstract: In this scientific work, the process of constructing a mathematical model of morphogenesis and dynamics of the number of bacteria that clean up oil pollution is considered. The creation of the model and the dedicated accompanying software are extremely important in such studies. Automation of processes makes it possible to predict the expected results and use various mathematical conditions and models in practice, in the field of oil pollution.

1 INTRODUCTION

Preparation of Software. The software for visualizing the mathematical model of the morphogenetic development of the studied microorganisms was developed using the Qt Creator integrated software development environment in C++ using the Qt library set (Blagodatsky, 1998).

The choice in favor of this development environment is due to its cross-platform nature - the ability to create software that is compatible with various operating systems (Windows, Linux, macOS, Android, etc.), the rich functionality of the built-in set of libraries and the wide possibilities in the field of software rendering. Thus, it is possible to launch the created software package on a wide range of computer devices.

Such a feature of Qt as the use of APIs (Application Programming Interface) of the low-level operating system was also taken into account, which makes it possible for the software created with it to work as efficiently as the software that was developed for specific platforms by other development tools (Dalgaard, 2011).

An important factor in choosing a development environment is the ability to quickly develop a user interface. This is possible thanks to the Qt Designer visual interface editing tool integrated into Qt Creator (Pepper, 1995).

Study Materials. Cells of the strain A. globiformis AC1112 pass through two stages in their morphogenetic cycle of development: bacillus-coccus (Hesty, 2017). During the lag phase, which occurs approximately in the interval of 0-9 hours, the cells increase in size (cocci with a diameter of 0.6 to 0.8 μm; rod-shaped from 2.3 × 0.5 to 3.1 × 0.7 μm), gradually transforming from coccoid to rod-shaped forms, at the end of this stage, the appearance of V-shaped and branched forms can also be observed. In the exponential growth phase, which runs from 9 to 48 hours, cells, intensively dividing, decrease in size (branched forms from 5.0 × 3.2 μm to 4.6 × 2.9 μm, curved forms from 2.1 × 1 .0 to 2.1×0.7 μm) and show different branching. The stationary phase occurs at approximately 60 hours of cultivation. In this phase, the branched forms, breaking up, give the original coccoid forms, while the diameter of the emerging cocci continues to be approximately 0.9 microns (Linos, 2000).

Cells of the G. alkanivorans K9 strain in the lag phase (0-12 h) are coccoid; here the cells slightly increase in size (diameter from 0.5 to 1.1 microns). In the exponential phase (12-60 hours), as the cultures grow, the cells gradually transform into rod-shaped cells, intensively divide, which leads to a decrease in their size (rods - 2.3 × 0.9-1.6 × 0.6 μm) . Various branches are observed here, as well as V-shapes, curved forms (branched forms - 3.4 × 0.6-3.2 × 0.5 μm, curved forms - 2.1 × 0.7-2.0 × 0 .6 μm).

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Subsequently, in the course of reaching the stationary phase (about the 60th hour), the cells begin the reverse transformation of cells of various forms into the form of cocci.

2 MATERIALS AND METHODS

Establishing Correspondence between Real and Theoretical Cell Shapes. For the most realistic reflection of the morphology of microorganism cells by the software package, a method was used, which consists in the selection of proportionality coefficients between different parts of the cell or the entire cell, taking into account the experimental data on cell sizes (Appendix A, tables A.3-A.4) obtained in the course of the study. The location of the cells on the field and the relative position of the parts of the cell for complex (branched) forms in the software package occurs randomly (Kummer, 2019).

Correspondence of real and theoretical forms of cells created by means of the software package is shown in Figures 1-2 for A. globiformis and Figures 3-4 for G. alkanivorans.

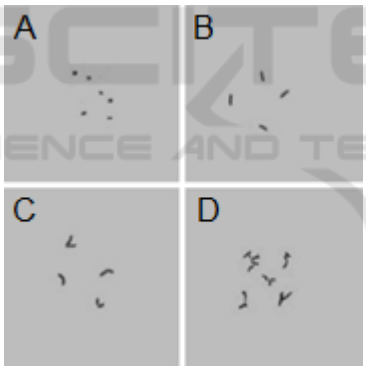

Figure 1: Natural forms of A. globiformis cells.

A - coccoid forms; B - rod-shaped forms; C - curved and V-shape; D - branched forms.

Description of the Mathematical Model. t is the life time of bacteria, determined by the formula (1):

$$t = H \times i, \tag{1}$$

where t is the lifetime of bacteria, h; H is the duration of the measurement period, h; i is the serial number of measurements in the experiment.

In this study, the value of H is a constant and equal to 3, the variable t varies between 0 and 44 (45 measurements in total), in accordance with the number of experimental measurements. At $t > 0$, the process of growth of microorganisms begins, and morphological changes in cells are observed (Ordoñez, 2021).

L is the cell length. W is the cell width. V is the volume of the cell: for cocci it is calculated according to the standard formula for finding the volume of a ball (formula (2)), and for rod-shaped ones, the formula for the volume of a cylinder is used (formula (3)), where the length of the cell L acts as the height, and for complex forms (branched , V-shaped), the volumes of individual rod-shaped branches are summed up (formula (4)). The radius for all types of cells (or their branches), except for cocci, is determined by formula (5).

$$V_S = \frac{4}{3}\pi R^3, \tag{2}$$

where V_S is the volume of the coccoid cell, $мкм^3$; R is the cell radius, $мкм$.

$$V_C = \pi R^2 L, \tag{3}$$

where V_C is the volume of a rod-shaped cell, $мкм^3$; R is the cell radius, $мкм$; L is the cell length, $мкм$.

$$V_M = \sum V_p, \tag{4}$$

where V_M is the volume of complex-shaped cell, $мкм^3$; V_P is the volume of the rod-shaped branch of a complex-shaped cell, $мкм^3$.

$$R = \frac{W}{2}, \tag{5}$$

where R is the cell radius, $мкм$; W is the cell width, $мкм$.

At each stage of morphogenesis, the average total number of cells N was calculated in ten fields of view (formula (6)):

$$N = \frac{\sum N_i}{10}, \tag{6}$$

where N is the average total number of cells; N_i is the total number of cells in one field of view.

In the same way, for ten fields of view, the average number of cells of individual forms n_x was calculated (formula (7)):

$$n_x = \frac{\sum n_i}{10}, \tag{7}$$

where n_x is the number of cells of a given form; n_i is the number of cells of a given shape in one field of view.

The quantitative proportion of cells of this form is determined according to the formula (8):

$$v_x = \frac{n_x}{N}, \quad (8)$$

where v_x is the quantitative proportion of cells of a given form, %; N is the average total number of cells in the field of view; n_x is the average number of cells of a given shape in the field of view.

The volume fraction of cells of this form is calculated by the formula (9):

$$\varphi_x = \frac{v_x}{\sum V_i}, \quad (9)$$

where φ_x is the volume fraction of cells of a given shape, %; V_x is the volume of a cell of a given shape, $мкм^3$; V_i is the volume of a cell of a separate form in a series, $мкм^3$.

The coefficient L_x, which reflects the volumetric and quantitative ratio between individual cell forms, is determined by formula (10):

$$L_x = \frac{v_i \varphi_i}{\sum v_i \varphi_i}, \quad (10)$$

where L_x is the linking coefficient; v_x is the quantitative fraction of a cell of a given shape, %; v_x is the quantitative proportion of cells of individual forms in the series, %; φ_x is the volume fraction of cells of a given form, %; φ_i is the volume fraction of cells of individual forms in the series, %.

The value d_x has been introduced, which links the dynamics of changes in the process of growth and development, both quantitatively and qualitatively - this is an indicator of the partial optical density for cells of a certain shape at a point in time. It is calculated for each type of cell shape individually. This value is determined by formula (11):

$$d_x = D_i L_x, \quad (11)$$

where d_x is the partial optical density; D_i is the optical density of cell culture at this stage; L_x is the linking coefficient.

Based on the principle of optical density additivity, the unknown optical density of the cell culture D can be calculated by adding the known partial optical densities (formula (12)).

$$D = \sum d_i, \quad (12)$$

where D is the optical density of the cell culture; d_i is the partial optical density of cells of individual forms.

Having data on the initial values of the number of cells N_0 and the optical density of the cell culture D_0, it is possible to calculate the number of cells at a given stage of growth for a given form of cells at a given stage of morphogenesis (formula (13)).

$$n_x = \frac{N_0 D}{L_x D_0}, \quad (13)$$

where n_x is the number of cells of a given form; D_0 is the initial optical density of the cell culture; D is the optical density of cell culture at this stage.

In the same way, the total number of cells is the sum of the number of individual cell shapes (formula (14)), known in advance or calculated using the above computational model constructs.

$$N = \sum n_i, \quad (14)$$

where N is the total number of cells; n_i is the number of cells of individual forms.

Determination of the Dependence of the Rate of the Course of the Morphogenetic Cycle of Development of Microorganisms on the Temperature of the Environment. In the framework of studies on the development of microorganisms, it often becomes necessary to establish the dependence of the growth rate on temperature. In modeling the processes of growth and development of bacterial cells, the equations of linear dependence have proven themselves very successfully. Here, the function y=k(T) is used, where k is an absolute indicator that characterizes growth at a certain temperature of the medium. In most cases, it is calculated from the initial and final number of cells; however, in this work, instead of these parameters, we resorted to using the initial and final optical density (formula (15)). The determining value in equations of this type is the angular coefficient of the straight line, which characterizes the dynamics of the change in the value of k under different temperature conditions of growth.

$$N = \sum n_i, \quad (15)$$

where k(T) is the absolute dependence of the MO growth rate on temperature, h-1; Dt is the final OD of cells; D0 is the initial OD of cells; tp is the duration of the logarithmic stage of growth, h.

The optimal temperature limits for growth for many coryneform bacteria are 20–30 degrees Celsius, so only these limits were considered in this work.

Since the most detailed morphological studies and related calculations were carried out at 30 °C, it is necessary to ensure that the value of the parameter k at a value of T = 30 °C is equal to one. To do this, we introduced a correction factor a (it has a unique numerical value for each microorganism), which will allow us to correct the model parameters taking into account the available experimental data, and obtained a computational design for calculating another parameter - the relative dependence of the growth rate of microorganisms on temperature - r(T) (formula (16)):

$$r(T) = a \times k(T), \qquad (16)$$

where $r(T)$ is the relative dependence of the *MO* growth rate on temperature, r^{-1}; a is the correction factor; $k(T)$ is an indicator of the absolute dependence of the *MO* growth rate on temperature, r^{-1}.

Calculation of Appropriate Models for Morphogenesis. Based on the experimental data obtained from the study of cells of bacterial strains, graphs were plotted, where the cultivation time was plotted on the abscissa axis, and the optical density was plotted on the ordinate axis, and growth curves were obtained (Figure 2).

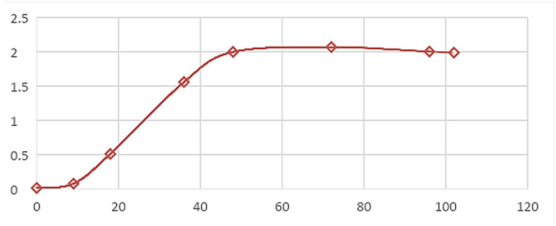

Figure 2: Globiformis growth curve at 30 °C.

In order to assess the contribution of cells of various shapes to the optical density, i.e., to calculate the partial optical density *d*, it was necessary to perform some intermediate calculations, namely: based on data on cell sizes, determine the volumes occupied by cells *V* (according to formulas (2) - (4)), volume fractions φ_x (according to formula (9)).

Having obtained the values of cell volumes *V* and then their volume fractions φ_x, and having known percentages of various cell shapes v_x, it is possible to determine the coefficient L_x by formula (10), and subsequently, according to formula (11), the partial optical density d_x for strain *A* (globiformis). Similarly, the corresponding values of the partial optical density d_x were calculated for strain *G.* (alkanivorans K9).

At the next stage, the obtained data were used to plot graphs that reflect the patterns of changes in cell morphology in the morphogenetic cycle of development of the studied bacterial strains, taking into account their contribution to the readings of optical density (Figures 3-4).

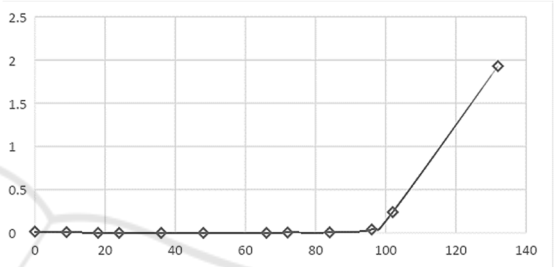

Figure 3: Dynamics of changes in partial optical density coccoid cell forms for A (globiformis, at 30°C).

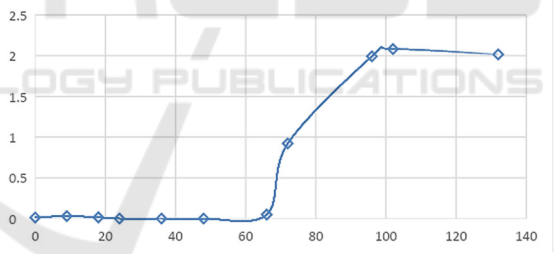

Figure 4: Dynamics of changes in partial optical density coccoid cell forms for G (alkanivorans, at 30°C).

For the convenience of work, the obtained graphs of the curve of the dynamics of changes in the partial optical density d_x for each culture, in turn, were divided into several time intervals (Perni, 2005). In addition to convenience, this was done to avoid overcomplicating features. And, based on their belonging to a certain type of charts, with the help of Microsoft Excel, a trend line and the corresponding approximating function were selected. In this case, the zero values of the functions were taken out of the graph, taking into account separately. The reliability coefficients for the approximation of R^2 functions have been brought to values as close as possible to unity in order to most accurately reflect the dynamics

of the described processes. Figures 4-6 show the intervals of the graph for coccoid forms of culture *A* (globiformis) with approximated functions, which subsequently become functions of the mathematical model, and calculated confidence factors.

Thus, the curve of the dynamics of changes in the partial optical density *d(t)* of culture *A* (globiformis) for coccoid forms is divided into segments 0-12, 72-105 and 108-132 hours (Figures 5-7). The first two curves are described by a polynomial type trend line, the last one by a linear type.

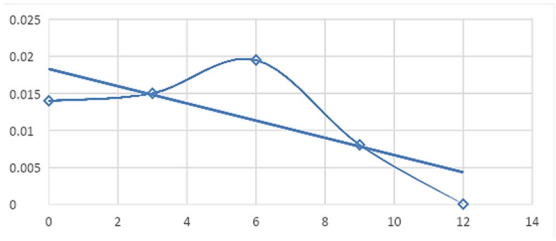

Figure 5: Curve and trend line for coccoid cell shapes A (globiformis between 0-12 hours, at 30°C).

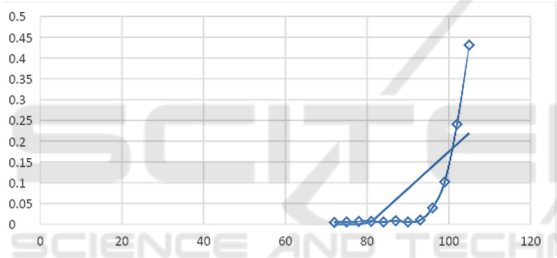

Figure 6: Curve and trend line for coccoid forms A (globiformis cells within 72-105 hours, at 30°C).

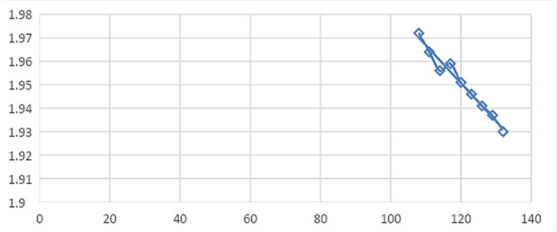

Figure 7: Curve and trend line for coccoid cells of A (globiformis between 108-132 hours, at 30°C).

3 RESULTS AND DISCUSSION

As a result, by combining individual functions into a system, taking into account the zeros of the functions, an equation of the model for the morphogenesis of microorganisms was obtained for the mathematical model developed within the framework of this study. Culture A (globiformis) corresponds to formula:

$$d(t) = \{0,0002t^4 - 0,00048t^2 + 0,00326t^2 - 0,00565t\}$$
$$t \in [0;\ 132]$$

where *t* is the lifetime of bacteria.

Thus, the growth of microorganisms depends on their life cycle, and the longer it is, the higher their efficiency in the treatment of oil pollution. In addition, it should be noted that additional factors affecting their effectiveness in terms of cleaning can be such parameters as temperature, pressure and stimulants, inhibitors, and so on. The construction of a mathematical model is very significant, since it allows the use of various software systems, which were discussed earlier (Magomedov, 2022). There are special libraries in the C++ programming language that have built-in methods for working with mathematical models and their visualization. Such a solution is also intended to speed up laboratory research, which can sometimes take a long time.

4 CONCLUSIONS

In conclusion, we can say that the morphogenetic development of A. globiformis AC1112 and G. alkanivorans K9 strains was studied: they are represented by the bacillus-coccus cycle and growth curves characterizing the increase in cell biomass (Moussa, 2020).

The physical dimensions of the cells during their growth, the diameter of A. globiformis cocci and G. alkanivorans cocci were determined; sizes of rod-shaped, branched-shaped, curved and V-shaped. The dependence of the growth of microorganisms on the temperature conditions of cultivation, the content of biogenic elements in the oil sludge medium and their relationship with other parameters of the processes under study has been established.

A mathematical model of morphogenesis and population dynamics of A. globiformis and G. alkanivorans in the process of oil pollution clean-up has been created. On its basis, software was considered and tested that allows determining the growth stage based on input data on the temperature of the cultivation medium and the content of biogenic elements in oil sludge, as well as visualizing the morphology of microorganisms. The model can serve as a tool for optimizing the temperature and chemical parameters of the growth environment of oil degrading bacteria, as well as increasing the efficiency of control during biological treatment.

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