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The Evolution Model of Cultured Bacteria with External Growth Inhibition: Computational Techniques and *in silico* Studies

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Abstract

Microbiological systems have become relevant objects of interdisciplinary research in mathematical biology and bioinformatics and can be analyzed using *in silico* studies with the implementation of computational experiments. The design of mathematical models of bacterial biomass growth under external inhibition can help to predict states of the microbiological system, reduce antibiotic use, and avoid antimicrobial resistance. The paper is devoted to developing a mathematical model of nutrient-dependent dynamics of bacteria cultured in media, considering external surface growth inhibition. The mathematical problem statement includes governing equations to define spatial-temporal distributions of bacterial biomass concentration, nutrient concentration, and time-dependent dose of antibiotic concentration. We propose a joint numerical scheme based on finite difference methods and specialized program application implemented with Matlab. A series of computational experiments were performed to describe the distributions of the key chemical compounds characterizing bacterial surface growth exposed to antibiotics and predict antibiotic treatment strategies.

1 Introduction

At present, it is accepted that bacteria are more than the sum of their parts: these microbes are able to exhibit complex, collective community behavior. The study of bacterial growth in nutrient media is one of the basic techniques used in microbiology, offering insights into

*Provided methodology, developing model, writing – original draft
†Designed algorithm and implemented software, validation, visualization
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bacterial behavior, environmental adaptations, and responses to external stressors such as antibiotics. Bacteria, as integrated communities of unicellular organisms, possess unique traits and demonstrate complex interactions with their environment, including nutrient uptake, metabolic activity, and colony formation [18].

Bacterial life processes are affected by various factors, including the presence of inhibitory substances like antibiotics. Antibiotics are agents that either kill bacteria (bactericidal) or inhibit their growth (bacteriostatic), and their application has been critical in managing bacterial infections. However, the emergence of antibiotic resistance necessitates a deeper understanding bacterial growth dynamics and the impact of antibiotics [1].

This research problem can be supported by mathematical modeling and *in silico* studies based on implementing computational experiments. Mathematical modeling is crucial for generating hypotheses, supporting experimental research, and advancing biological control methods [19]. Computational techniques are well-suited for analyzing nonlinear behaviors in biological systems, from individual microorganisms to entire communities.

Overall, microbiology has indicated that bacterial communication underlies the abilities of these microbes to interact using special chemical signals and coordinate the group activities [6, 11]. Bacterial communication regulates virulence, host interactions, and microbial growth, contributing to bacterial resistance against antibacterial agents. Mechanisms like chemotaxis, genetic exchange (plasmids), and quorum sensing are central to this process [2]. Over the past two decades, bacterial quorum sensing has been extensively explored using mathematical models based on various approaches, such as differential equations, Monte Carlo simulations, cellular automata, particle modeling, etc. These achievements are critical in understanding the dynamics of bacterial colonies grown in nutrient media and biofilm formation [10, 15, 33, 22].

Biological experiments often observe bacteria in biofilms, which offer a structured environment for growth and survival [3, 4], or in laboratory cultures on nutrient substrates [28, 27]. The biocompositions of the cultivated bacterial culture and biofilm are very similar, but the geometry of the structures is quite different. In this way, various approaches are used for modeling bacterial communities, such as the deterministic differential apparatus [7, 30] as well as discrete-dynamical simulations [5, 21, 26]. In many cases, these models can help in tracking microbial processes and identifying infectious agents. To be more precise here, we mention some prime and most useful approaches. The Monod [16] and Droop [31] models can be applied for modeling microbial population dynamics, assuming that spatial distribution is not relevant (these models are formalized by ordinary differential equations). In the view of modeling spatial dynamics, the reaction-diffusion approximation can be used provided by the Fisher or Allen-Cahn models [12, 20], and various modifications to visualize bacterial growth in nutrient media and the development of biofilms [8, 14].

Nevertheless, despite the many available approaches, there is no single theory or model that can describe all bacterial structures. Each unique bacterial culture requires selecting the most suitable model and adjusting its parameters to accurately depict natural patterns. Different bacterial strains, when cultivated under specific conditions (such as humidity, pH, oxygen levels, temperature, and nutrients), form diverse self-similar structures, including spirals, meanders, explosive forms, and branching dendrites [29]. A key aspect of modeling these patterns is incorporating mechanisms that allow the formation of irregular, fractal, or self-affine structures resulting from self-organization. Previously, a hybrid method, combining differential equations with a stochastic procedure had been proposed for better simulations of naturalistic irregular patterns of bacterial growth [14].

Specification of the strategies of antibiotic treatment is of particular importance in the direction of pathogen inhibition. Overall, in this case, there are relevant subproblems to developing

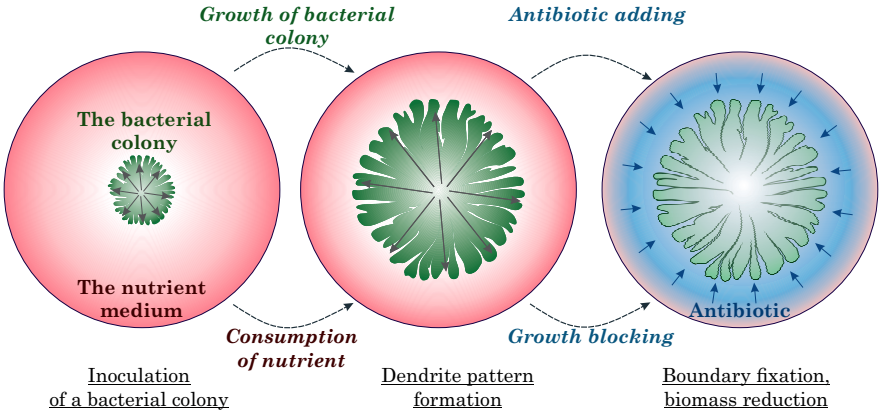

Figure 1: The schematic representation of the successive stages for biological process.

a model that informs antibiotic treatment strategies by predicting the effects of different dosing regimens on bacterial growth. This tool intends to optimize treatment protocols, reduce antibiotic resistance, and improve patient outcomes, which is increasingly essential as resistance rises.

In our previous study [15], we have proposed a model that describes the dependence of substances that characterize quorum sensing in bacterial communities under antibiotic action. An important stage of such research is the approximation of the spatio-temporal configuration of bacterial biomass. Previously, we used geometric primitives, the logistic growth law, and a stochastic algorithm to specify bacterial dynamics. The main contribution compared to previous studies is represented by the concept of developing a generalized model of bacterial pattern formation (corresponding to naturalistic scenario of the bacterial colony evolution) under the external condition of multiple antibiotic treatment. Hence, in the current study, we formalize a reaction-diffusion mathematical model of nutrient-dependent dynamics of bacteria cultured in media, derive a numerical scheme and computation algorithm taking into account the regime of external inhibition, and perform a series of computational experiments to visualize spatial-temporal evolution of biomass.

2 Problem statement

In this section, we give a brief overview about the necessary biological background and introduce the mathematical model equations.

2.1 Brief biological setup

Let us start with a conceptual formulation of the problem of modeling bacterial growth during surface cultivation in a nutrient medium (for example, one can use agar in Petri dishes). Figure 1 shows the simplified scheme of evolution of the biological system.

We assume that only one colony of a predefined bacterial culture is isolated and planted. After inoculation, one can observe the formation of a dendrite bacterial pattern as a result of nutrient consumption by bacteria. As the basic mechanisms, we will consider the dynamics, diffusion, and reaction of the “competing” processes of changing the concentrations of bacteria and nutrients. Bacterial growth in this approximation is determined only by the available level

of nutrient, initial and boundary conditions. The reaction component generally corresponds to the representation of Michaelis-Menten kinetics.

In addition, we suppose that at a certain time point, a dose of antibiotic was added to the Petri dish and distributed evenly over the surface. Notably, biological experiments suggest that antibiotic adding leads to the blocking of biomass growth, fixation of boundaries of bacterial patterns, and reduction of biomass. Also, let us simplify the problem - we will consider the growth of the bacterial colony in a two-dimensional area. In this regard, the problem can be formulated as follows. It is required to determine the spatio-temporal dynamics of the concentration of biomass and nutrient substrate over time, taking into account the inhibition process.

2.2 Governing equations

In the framework of the present study, we modify a basic nutrient-dependent bacterial growth model proposed in [14]. To be specific, we will consider a two-dimensional computational domain. The mathematical model statement is expressed by an initial-boundary value problem for nonlinear time-dependent reaction-diffusion equations in normalized view:

$$\frac{\partial n}{\partial t} = D_n \Delta n - v_n n b, \quad x \in \Omega, \quad t \in (0, T], \tag{1}$$

$$\frac{\partial b}{\partial t} = \nabla (D_b \nabla b) + v_b n b, \quad x \in \Omega, \quad t \in (0, T], \tag{2}$$

$$n(x, 0) = n_0, \quad b(x, 0) = b_0(x), \quad x \in \Omega, \tag{3}$$

$$\frac{\partial n}{\partial \mathbf{n}}\Big|_\Gamma = 0, \quad \frac{\partial b}{\partial \mathbf{n}}\Big|_\Gamma = 0, \quad t \in (0, T), \tag{4}$$

where $n(x, t)$ is the nutrient concentration; $b(x, t)$ is the bacterial biomass concentration; D_n, v_n, v_b are positive parameters; the diffusion coefficient of the bacterial mass depends on the nutrient $D_b = \sigma n b$, $\sigma = \sigma_0(1 - \delta)$, where σ_0 is a parameter characterizing the concentration of nutrient and δ is the random variable characterizing biomass fluctuations during the dynamics of colonies, $|\delta| < 1$; $\mathbf{n}$ is the outward normal vector to the boundary Γ; T defines the time range of process observation.

Further, we assume that at a certain time point t_A, an antibiotic dose is added to the entire Petri dish. In this case, the colony's growth will almost immediately cease, and the antibiotic will gradually begin to kill the existing bacteria. According to biological data, we should include the corresponding mechanisms in the computation algorithm. Furthermore, the intensity of the antibiotic effect is assumed not to be constant: after the addition, it increases, and then, after reaching maximum effectiveness, it decreases again.

This implies that we transform the original system (1)–(4) in such a way as to take into account the described changes. After the time t_A, the growth and movement of bacteria stop, so in equation (2) we need to remove the terms responsible for diffusion and growth of the bacterial mass due to nutrient consumption and add a term responsible for killing bacteria with the antibiotic. To approximate this term, we use the form proposed in [25]:

$$g(x, t) = -\frac{E_{max} A(t)}{A_{50} + A(t)} b(x, t), \tag{5}$$

where A is the time-dependent intensity of the antibiotic effect related to an usual exponential decay according to natural pharmacodynamics; E_{max} is the maximum killing rate of bacteria

by antibiotics; A_{50} is the antibiotic concentration at which the killing rate of bacteria is half maximal.

Also, we introduced a law to define the changes in antibiotic concentration. To be precise here, we consider the antibiotics of ciprofloxacin as an example [24]. We apply the Rayleigh distribution proposed in [15]:

$$A(t) = A_0 t\, exp\left(\frac{-t^2}{A_1}\right), \tag{6}$$

where A_0, A_1 are the approximation parameters. Furthermore, since the effect of the nutrient is no longer taken into account after the addition of the antibiotic assuming there is no further bacterial growth or movement, formalized in the following by the characteristic function $\chi_{t\leq t_A}$), the equation (1) can be excluded from the calculations when $t \geq t_A$.

Based on the above, we can rewrite the system (1)–(4) in accordance with our assumptions about the effect of the antibiotic:

$$\frac{\partial n}{\partial t} = D_n \Delta n - v_n n b \chi_{t\leq t_A}, \quad x \in \Omega, \quad t \in (0, T], \tag{7}$$

$$\frac{\partial b}{\partial t} = (\nabla(D_b \nabla b) + v_b n b)\chi_{t\leq t_A} - \frac{E_{max} A}{A_{50} + A} b, \quad x \in \Omega, \quad t \in (0, T], \tag{8}$$

$$A(t) = 0, \quad t \in [0, t_A), \tag{9}$$

$$A(t) = A_0 (t - t_A)\, exp\left(\frac{-(t - t_A)^2}{A_1}\right), \quad t \in [t_A, T]. \tag{10}$$

The initial and boundary conditions (3)–(4) should be also imposed. Let us note that in this study we have made several simplified assumptions in order to explore antibiotic inhibition regimes for bacterial patterns growing in nutrient medium. Here we suppose that the dose of antibiotics covering the culture is sufficient to completely stop the growth of the colony along the colony boundary. We do not take into account the diffusion of antibiotics in the Petri dish. In addition, the two-dimensional model limits the consideration of the colony only on the surface (indeed, the nutrient consumption and reproduction by the surviving part of the colony could continue in depth).

3 Numerical tools, computational experiments and discussion

Next, we introduce the numerical procedures and the results we can gain from them.

3.1 Computational scheme

Constructing analytical solutions to nonlinear reaction-diffusion problems causes particular difficulties. Therefore, in the practice of applied modeling, numerical methods play a priority role. Within the framework of this study, the finite-difference splitting method is used.

The computational scheme and algorithm are based on the finite difference method using the Yanenko fractional step approach [32]. First, we construct a uniform grid on the 2D computational domain as follows:

$$\Omega^{\tau}_{h_x,h_y} = \left\{x_i = -L + ih_x,\, y_j = -L + jh_y,\, t_k = k\tau,\, i = \overline{0, N},\, j = \overline{0, M},\, k = \overline{0, K}\right\}. \tag{11}$$

This yields that we can introduce the grid functions $n_{i,j}^k$ and $b_{i,j}^k$ corresponding to the values of the functions $n(x,y,t)$ and $b(x,y,t)$ at the nodes (x_i, y_j, t^k), respectively. As known, the Yanenko scheme is based on splitting each temporal step into two half-steps along the x and y coordinates respectively. The scheme is characterized by the accuracy order $O(h_x^2+h_y^2+\tau)$. In addition, due to the presence of reaction terms, we also supplement the computational scheme with an iterative procedure to support the accuracy order of approximation with respect to time.

At each half-step in time we will take into account the derivative only with respect to one spatial coordinate (x or y); at the first half-step $k+1/2$ we will have the following approximation of the original equations (1) and (2):

$$\frac{n_{i,j}^{(s)} - n_{i,j}^k}{\tau} = \frac{n_{i+1,j}^{(s)} - 2n_{i,j}^{(s)} + n_{i-1,j}^{(s)}}{h_x^2} - \frac{1}{2}F_{i,j}^{(s)}, \tag{12}$$

$$\frac{b_{i,j}^{(s)} - b_{i,j}^k}{\tau} = \sigma n_{i,j}^{k+1/2} b_{i,j}^{(s-1)} \frac{b_{i+1,j}^{(s)} - 2b_{i,j}^{(s)} + b_{i-1,j}^{(s)}}{h_x^2} + \\ + \sigma \left(\frac{n_{i+1,j}^{k+1/2} - n_{i-1,j}^{k+1/2}}{2h_x} b_{i,j}^{(s-1)} + n_{i,j}^{k+1/2} \frac{b_{i+1,j}^{(s-1)} - b_{i-1,j}^{(s-1)}}{2h_x} \right) \frac{b_{i+1,j}^{(s)} - b_{i-1,j}^{(s)}}{2h_x} + \frac{1}{2}F_{i,j}^{(s)}, \tag{13}$$

where $i = \overline{1, N-1}$, $j = \overline{1, M-1}$ and $s = 1, 2, ...$ is the iteration number.

At the $k+1/2$ time layer $\{n_{i,j}^{(s)}\} \to n_{i,j}^{k+1/2}$, starting from $n_{i,j}^{(0)} = n_{i,j}^k$, and $\{b_{i,j}^{(s)}\} \to b_{i,j}^{k+1/2}$, starting from $b_{i,j}^{(0)} = n_{i,j}^k$. The reaction term in the equation for nutrient distribution (12) is given by $F_{i,j}^{(s)} = b_{i,j}^k n_{i,j}^{(s)}$, then the equation for bacterial mass (13) is solved with $F_{i,j}^{(s)} = n_{i,j}^{k+1/2} b_{i,j}^{(s)}$, and then refined in (12) with $F_{i,j}^{(s)} = b_{i,j}^{k+1/2} n_{i,j}^{(s)}$.

At the second half-step $k+1$ the scheme is expressed as follows:

$$\frac{n_{i,j}^{(s)} - n_{i,j}^{k+1/2}}{\tau} = \frac{n_{i,j+1}^{(s)} - 2n_{i,j}^{(s)} + n_{i,j-1}^{(s)}}{h_y^2} - \frac{1}{2}F_{i,j}^{(s)}, \tag{14}$$

$$\frac{b_{i,j}^{(s)} - b_{i,j}^{k+1/2}}{\tau} = \sigma n_{i,j}^{k+1} b_{i,j}^{(s-1)} \frac{b_{i,j+1}^{(s)} - 2b_{i,j}^{(s)} + b_{i,j-1}^{(s)}}{h_y^2} + \\ + \sigma \left(\frac{n_{i,j+1}^{k+1} - n_{i,j-1}^{k+1}}{2h_y} b_{i,j}^{(s-1)} + n_{i,j}^{k+1} \frac{b_{i,j+1}^{(s-1)} - b_{i,j-1}^{(s-1)}}{2h_y} \right) \frac{b_{i,j+1}^{(s)} - b_{i,j-1}^{(s)}}{2h_y} + \frac{1}{2}F_{i,j}^{(s)}, \tag{15}$$

where $i = \overline{1, N-1}$, $j = \overline{1, M-1}$ and $s = 1, 2, ...$ is the iteration number.

At the $k+1$ time layer $\{n_{i,j}^{(s)}\} \to n_{i,j}^{k+1}$, starting from $n_{i,j}^{(0)} = n_{i,j}^{k+1/2}$, and $\{b_{i,j}^{(s)}\} \to b_{i,j}^{k+1}$, starting from $b_{i,j}^{(0)} = n_{i,j}^{k+1/2}$. The reaction function in the nutrient equation (14) is given by $F_{i,j}^{(s)} = b_{i,j}^{k+1/2} n_{i,j}^{(s)}$, then the bacterial mass equation (15) is solved with $F_{i,j}^{(s)} = n_{i,j}^{k+1} b_{i,j}^{(s)}$, and then refined in (14) with $F_{i,j}^{(s)} = b_{i,j}^{k+1} n_{i,j}^{(s)}$.

The scheme is supplemented by a finite-difference approximation of the boundary conditions (4). The values of $b_{i,j}^0$ and $n_{i,j}^0$ are set based on the initial conditions of the problem (3). All obtained systems of linear equations were solved by the sweep method while controlling the accuracy of the iterative processes as well as the practical convergence of the computational scheme.

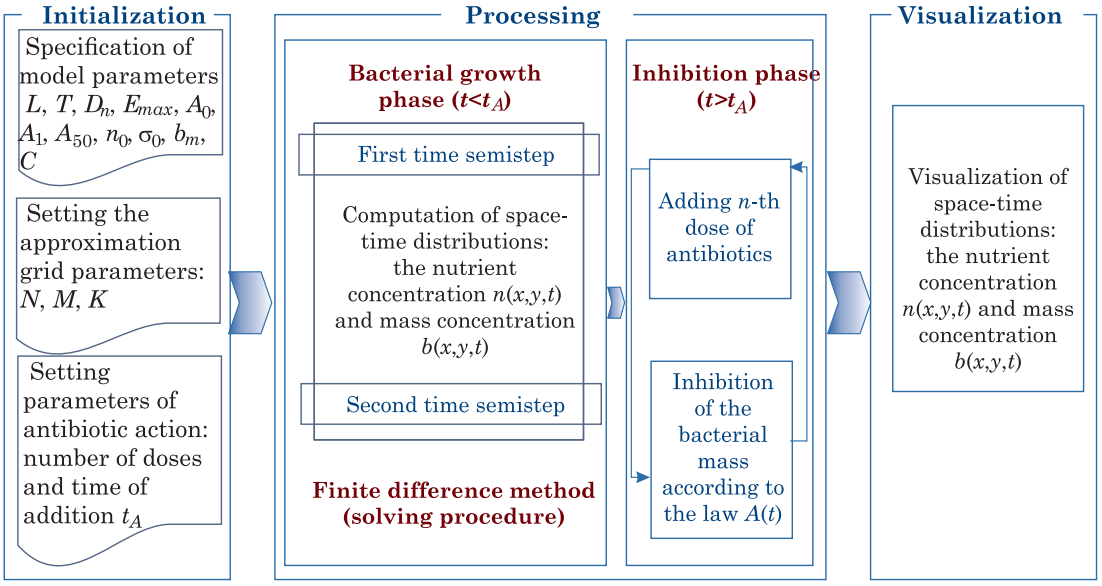

Figure 2: Functional diagram of model implementation.

3.2 Program implementation aspects

The algorithm for the numerical solution was implemented as an application program using Matlab software. The functional diagram of the simulation procedure is illustrated in Fig. 2.

The initialization block includes setting the parameters of the biomass growth model and antibiotic dynamics, as well as the mesh parameters. The main processing part of the program includes two time cycles, respectively, before and after the moment of inhibition. Before adding the antibiotic, nutrient concentration is calculated and dendrite growth of the bacterial culture is simulated. After the moment of antibiotic treatment, computation of nutrient concentration is not performed. The spatial-temporal distribution of biomass as well as the integral characteristic - the total concentration of biomass B - are calculated. The post-processing block includes visualization of the results of calculating the spatial-temporal characteristics of the biosystem.

3.3 Simulation results

To implement numerical experiments, we defined a set of model parameters. Following to the normalized formalization of the model, we perform all computations using arbitrary units (a.u.) for defined characteristics as well as model parameters: $L = 250$ a.u., $T = 3000$ a.u., $D_n = 1$ a.u., $E_{max} = 0.05$ a.u., $A_{50} = 0.073$ a.u., $A_0 = 0.02$ a.u., $A_1 = 20$ a.u., $n_0 = 0.5$ a.u., $\sigma_0 = 1$ a.u., the random parameter δ was calculated at each approximation node using a triangular distribution in the range $(-1, 1)$. Also, we apply a function to approximate an initial bacterial colony distribution at the center of the computational domain:

$$b_0(x, y) = b_m \exp\left(-\frac{x^2 + y^2}{C^2}\right),$$

where $b_m = 0.71$ a.u. and $C^2 = 6.25$ a.u.

Moreover, we conducted simulations for double antibiotic treatment: at the moment $t_{A_1} = 2500$ a.u. and $t_{A_2} = 2750$ a.u. The latter allowed us to simulate the gradual inhibition of the bacterial biomass using several doses of the antibiotic. To measure the effectiveness of the antibiotic effect at each step of time, the total volume of bacterial mass was calculated using the following formula:

$$B = \sum_{i=0}^{N} \sum_{j=0}^{M} b_{i,j},$$

where $b_{i,j}$ are the values of bacterial mass concentration in the node (i, j).

By the above assumption, the concentration dynamics for ciprofloxacin approximates experimental observations: the maximum value reaches 4-8 hours and after approximately 12-24 hours the concentration drops to a certain level [24].

The main goal of the simulations was to visualize naturalistic patterns of bacterial cultures during their evolution as well as external exposure to antibiotics. Figure 3 shows the simulation result for the formation of a dendrite bacterial pattern with the process of simultaneous nutrient consumption at fixed times.

The changes in bacterial biomass concentration are illustrated in Fig. 4. Our observations correspond to mechanisms underlying the model and experimentally observed behavior. The antibiotic adding leads to stopping of growth of bacterial patterns and essentially reducing biomass. However, after the antibiotic has finished acting, the changes in bacterial mass cease. We indicate a similar effect due to the second dose of antibiotics.

Since predicting the effectiveness of an antibiotic for its given characteristics is important, we also assessed the integral characteristic. Figure 5 demonstrates the dynamic changes in the total value of biomass concentration B under double antibiotic treatment at intervals of $t = 250$ a.u. The simulation results suggest that the "active" phase of antibiotic action lasts approximately 100 a.u., during which the total value of biomass concentration decreases sharply by almost 50%. If the biomass concentration is rather high, then to achieve a therapeutic effect, repeated inhibition is required to destroy a significant part of the population.

The specific behavior of the biomass characteristic (missing possible growth after antibiotics application) is due to the assumed mechanisms underlying the model, namely, a sufficient amount of antibiotics to stop the growth of the colony and consideration of the characteristics of the colony only on the surface of the Petri dish (without taking into account the biological processes of antibiotic diffusion, consumption of the nutrient and reproduction of the surviving part colonies in depth on the lower substrate). Although this version of the implemented model has some limitations, we can use the obtained data for appropriate experimental conditions and further modification of the computational approach. Therefore, the prospect of the current study can be represented by extension of the model to a three-dimensional analogue.

4 Conclusions

Finally, a modification of a two-dimensional mathematical model was proposed, and computational techniques were designed to estimate nutrient-dependent dynamics of bacteria cultured in the medium in view of the regime of inhibition by antibiotics. We supplemented a finite difference scheme related to the numerical solving reaction-diffusion problem by the numerical approximation of the concentration dynamics for typical antibiotics and the time dependence of biomass on antibiotic concentration. We implemented a numerical algorithm as an applied program using Matlab. The program is indented to calculate nutrient and biomass distributions under variation of inhibition regimes (intensity, duration of effect and number of doses of the

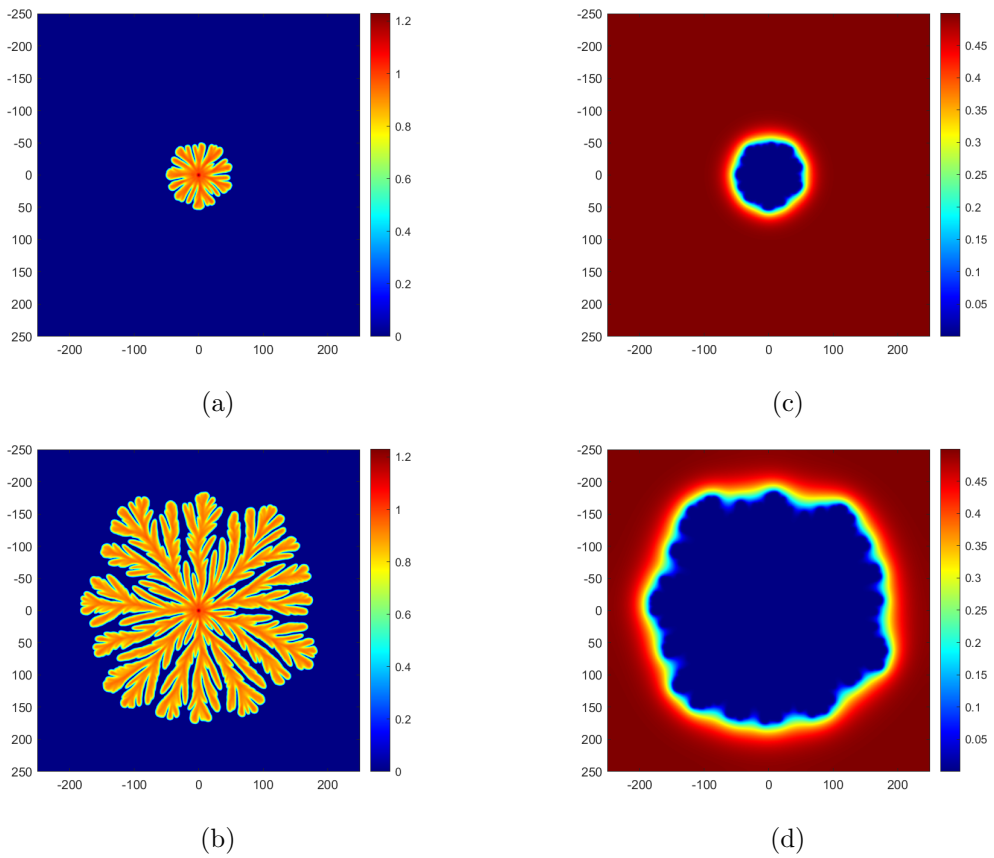

Figure 3: The spatial distributions of bacterial biomass concentrations – (a), (b) and nutrient concentration – (c), (d) at fixed time moments: $t = 500$ a.u. – (a), (c), $t = 2500$ a.u. – (b), (d).

antibiotic, number of doses). A series of performed numerical experiments (at certain values of simulation parameters) allowed us to indicate that although the antibiotic blocks colony growth, a significant reduction in biomass (for example, 10 times) can only be achieved as a result of repeated inhibitions. Moreover, the issue of using low doses of antibiotics, which can lead to the resumption of colony activity in the presence of nutrient, requires a separate study.

The important contribution of the present study is represented by the modification of the 2D model of nutrient-dependent bacterial growth in the case of dynamical inhibition of biomass by antibiotic treatment. Such models provide the foundation for *in silico* prediction of the behavior of pathogenic bacteria under degradation conditions. Our efforts are aimed at further improving this approach by taking into account bacterial quorum sensing, which is controlling bacterial resistance. Developments in a 3D hybrid model of bacterial population evolution, incorporating mechanisms that regulate resistance against antibiotics through quorum sensing, can provide deeper insights into the complex behavior of bacterial communities. At the present stage of art, the question regarding the justification and mathematical formalization of such mechanisms still remains open and requires further investigations.

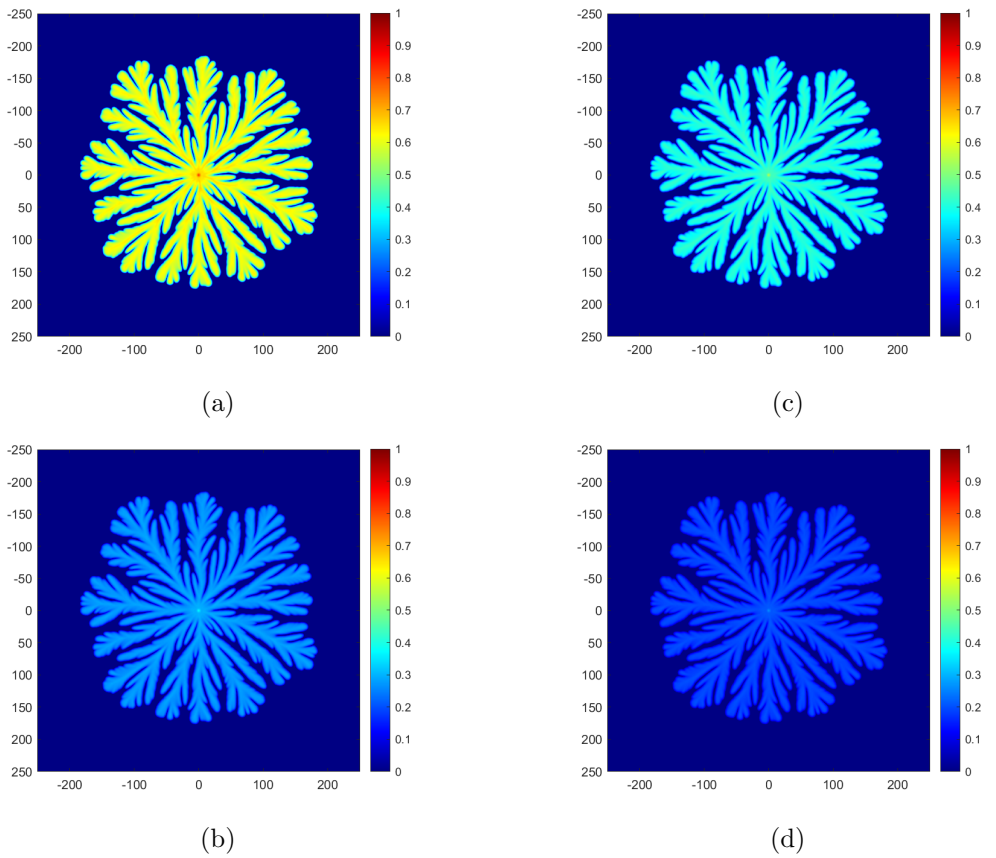

Figure 4: Consecutive stages of biomass distribution during double inhibition by an antibiotics: $t = 2530$ a.u. – (a), $t = 2750$ a.u. – (b), $t = 2780$ a.u. – (c), $t = 2810$ a.u. – (d).

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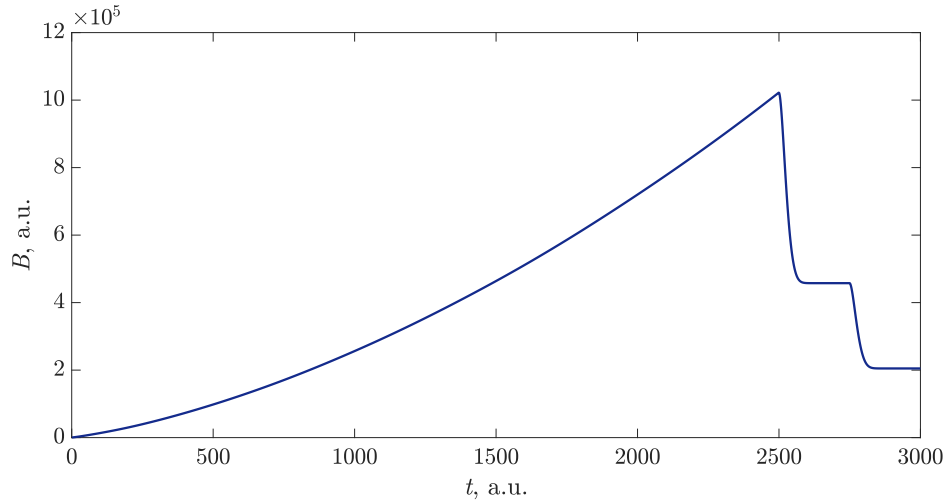

Figure 5: The time dependence of total value of bacterial biomass during double antibiotic action.

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