RESEARCH ARTICLE

# Biofilm quorum sensing model based on differential equation mathematical model

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**The current methods for analyzing biofilm quorum sensing cannot analyze the time and space of dynamic data, resulting in low accuracy in analyzing the process of biofilm quorum sensing. To solve this problem, this study used ordinary differential and partial differential equations to construct a quorum sensing model for biofilm to analyze the concentration of various substances in bacteria during biofilm quorum sensing. The study further analyzed the performance of the differential equation model. The results showed that the calculation accuracy of the proposed model was higher than 98% in different datasets, and the average computation time of the model was less than 1.5 seconds. Analysis of the biofilm quorum sensing process using this proposed model revealed that, during this process, the downregulation of biomass and nutrient concentrations within the biofilm was negatively correlated with time, whereas the upregulation of biomass and quorum sensing signal concentrations was positively correlated with time. The results of this study suggested that the proposed model could accurately analyze the concentration of substances during bacterial biofilm quorum sensing.**

**Keywords:** ordinary differential equations; partial differential equations; biofilm; quorum sensing model; substance concentration.

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## Introduction

Biofilm refers to an organized bacterial population that adheres to the surface of an object and is enveloped by extracellular macromolecules of bacteria [1]. There are various biomolecules such as proteins, deoxyribonucleicacid (DNA), ribonucleic acid (RNA), peptidoglycan, *etc*. in biological membranes. This structure can create stable internal conditions for cellular life activities, guide the connections between cells and the matrix, and undertake functions such as material transportation, information transmembrane transmission, and energy conversion [2]. Microorganisms or bacteria can secrete various sensing signaling molecules through themselves, which can coordinate various behaviors inside bacterial biofilms. Through the above process, microorganisms or bacteria can adapt to changes in the external environment, which is called the biofilm quorum sensing (BQS) process [3, 4]. BQS can play an important role in biofilm formation, biofilm functional regulation, and balance maintenance [5]. The important mechanism of quorum sensing is the generation, release, accumulation, and perception of signaling molecules including the concentration changes of upregulated biomass, downregulated biomass, nutrients, and quorum sensing signal analysis within the biofilm [6]. To help relevant personnel understand the substances required by microorganisms or bacteria in the process of BQS and coordinate the collective behavior of organisms, it is necessary to analyze the concentration of various substances inside the biofilm [7].

At present, many scholars have analyzed the process of quorum sensing in biofilms. Barani *et al*. used electromagnetic signal transduction methods to analyze the concentration of substances inside biofilms to analyze the quorum sensing effect in bacterial biofilms and reached the accuracy of 89.6% for BQS analysis [8]. Somathilaka *et al*. used a gene conditional neural network to analyze the inaccurate analysis of BQS in biological artificial intelligence. The results showed that the accuracy of this method in analyzing BQS effect reached 92.1%, but the analysis effect of this method on biofilm spatial data was poor [9]. In addition, to analyze the antibacterial effect during the process of BQS, Cimolato *et al*. used the Quenching strategy to construct a BQS process analysis model and found that the accuracy of this method in analyzing the antibacterial effect during the quorum sensing process reached 87.6%. Although its analysis effect was relatively good, it did not consider the changes in BQS over time [10]. For the issue of difficult analysis of chemical substances in the quorum sensing pathway in human immune defense, Zulfiqar *et al*. analyzed the communication molecules involved in this process and the chemical substances accordingly. The results suggested that this method had better analysis performance [11]. However, there are still problems in these studies such as poor analysis results and low analysis accuracy due to the inability to analyze dynamic data in both time and space simultaneously. Therefore, it is necessary to optimize the current analytical methods and improve the accuracy of time and space analysis of dynamic data using BQS analysis methods to ensure their effectiveness in analyzing the BQS process. Ordinary differential equation (ODE) can describe time-varying problems in bioinformatics engineering, as well as the laws governing how substances change over time [12, 13], while partial differential equation (PDE) can describe problems in spatial variations [14]. ODE and PDE are commonly used in various models to analyze material changes and movements. Li *et al*. utilized the ODE to predict stock market prices and found that its prediction accuracy reached 93.2% [15]. In addition, Nakai-Kasai *et al*. also used the ODE to process and analyze signals in wireless communication and found that the prediction error of this method was only 2.4% [16]. To analyze electromagnetic and acoustic information, Ji *et al*. constructed a PDE model for electromagnetic and acoustic waves and confirmed the accuracy of the model reaching 93.4% [17]. To predict the interference of planetary load-bearing fault layer signals, Wang *et al*. designed a signal prediction method using PDEs and found that the prediction error of this method was only 2.1% [18].

The current BQS models have poor performance and low accuracy in analyzing dynamic data in both time and space. To address these issues, this study combined ODE with PDE and used the Euler method to solve the model, obtaining the changing trends of various substance concentrations in the biofilm during the sensing process and coordinating the behavior of microbial communities accordingly. This study addressed the limitations of existing models, which struggled to simultaneously depict the temporal accumulation effect and spatial diffusion dynamics of quorum sensing signals. The proposed ODE-PDE model of this study filled the methodological gap in spatiotemporal multiscale coupled modeling and provided a theoretical basis for precise regulating biofilm formation and developing targeted antimicrobial strategies against quorum sensing, which held significant application prospects in the control of biofilm-related infections.

## Materials and methods

### Biofilm quorum sensing model based on ODE

This study used the ODE model to analyze the quorum sensing process of biofilms to analyze the functions of biofilms. The ODE model included an independent variable and an unknown function. Among them, the independent variables were time and spatial coordinates. The unknown function represented the function that needed to be solved such as the trend of the function over time. The first and second derivatives in the equation could reflect changes in the function. The first derivative could reflect changes in the velocity of an object, while the second derivative could reflect changes in the acceleration of a material. The order of the equation was determined by the order of the highest derivative. Solving the model involved solving the equation to obtain the final solution. The process of bacterial BQS involved synthesis, diffusion, degradation, and dynamic regulation of gene expression through signal analysis. Direct modeling would face multiple dimensions, and ODE could perform variable dimensionality reduction to simplify complex systems into solvable equation systems. When constructing a BQS model using ODE, the modeling process consisted of five steps including modular decomposition, variable dimensionality reduction, equation construction, parameter calibration, and dynamic simulation (Figure 1).

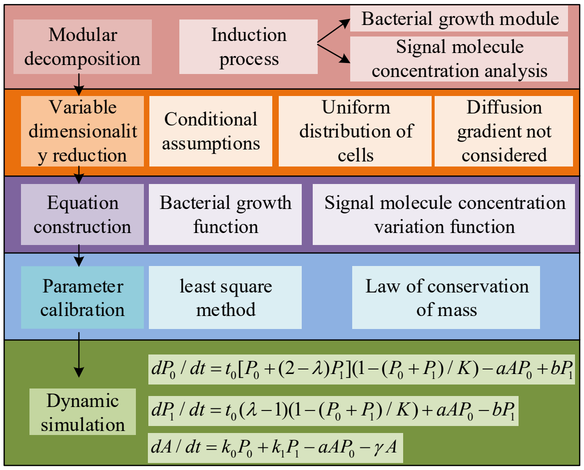


**Figure 1.** Modeling process of biofilm quorum sensing model for ODE.

In the modular decomposition process, the multi-scale interactions involved in the BQS process were first decomposed into modules that could be independently analyzed. Each module focused on a specific biological process, thereby transforming the biofilm sensing process into a function, allowing the BQS process to be expressed in a visual form. The process of BQS was mainly decomposed into the bacterial growth module and the BQS signal molecule concentration analysis module. Afterwards, ODE simplified the equation by making assumptions to reduce the dimensionality of high-dimensional variables. Assuming uniform distribution of cells within the biofilm, ignoring diffusion gradients, and assuming that the rapid equilibrium process reached a steady state, the equilibrium constant was directly used to represent the concentration of the complex, thereby reducing the number of differential equations. After reducing the dimensionality of variables, differential equations were constructed to obtain functional expressions for the bacterial growth module and the BQS signal molecular module. The parameters of the constructed function expression were then calibrated, and the least squares method was utilized to calibrate the model parameters. The expression for its calibration approach was shown below.

$$f(x) = \alpha 1\varphi 1(x) + \alpha 2\varphi 2(x) + \cdots + \alpha m\varphi m(x) \quad (1)$$

where $\alpha$ was undetermined coefficients. $m$ was data sequence number. $\varphi m(x)$ was a selected set of linearly independent functions. Based on the law of conservation of mass and calibration using the least squares method, the function expression for the module related to bacterial growth was constructed as follows.

$$\begin{cases} dP_0/dt = t_0[P_0 + (2-\lambda)P_1](1-(P_0+P_1)/K) - aAP_0 + bP_1 \\ dP_1/dt = t_0(\lambda-1)(1-(P_0+P_1)/K) + aAP_0 - bP_1 \end{cases} \quad (2)$$

where $P_0$ was the concentration of down regulated bacterial biomass during the process. $\lambda$ was the conversion rate of bacteria within the biofilm. $a$ was the degree of mutual conversion between up regulated and down regulated biomass. $b$ was the conversion coefficient. $P_1$ was the concentration of up regulated bacterial biomass during the process. $t_0$ was the time

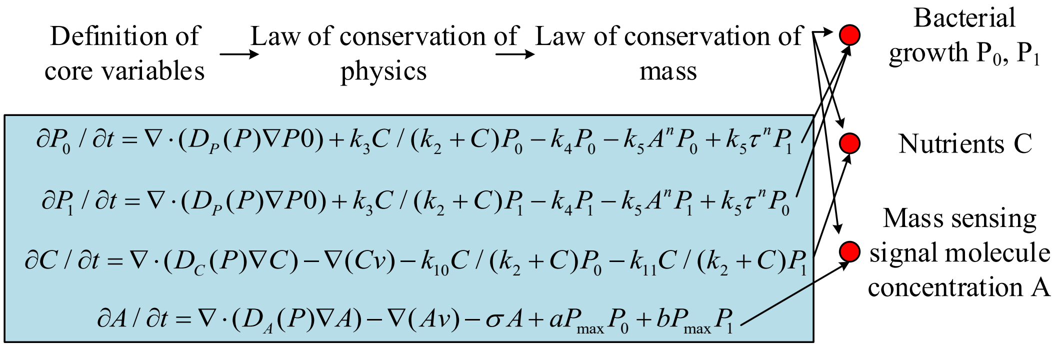


**Figure 2.** Population sensing model of biofilm based on PDE.

parameter that affected the bacterial classification speed during the BQS process. $K$ was the maximum value that the bacterial biofilm could withstand. $A$ was the concentration of signal analysis of BQS. $t$ was the bacterial growth time. The functional expression for the concentration analysis of BQS signal molecules constructed was shown below.

$$dA/dt = k_0 P_0 + k_1 P_1 - aAP_0 - \gamma A \tag{3}$$

where $\gamma$ was the decay rate of signal molecules in BQS. $k_0$ was the speed at which downregulated substances generated sensing signals in BQS. $k_1$ was the speed at which upregulated substances generated sensing signals in BQS. The calibrated function expression was eventually solved by using the Euler method to simulate the dynamic process of BQS and validate the model. The basic formula of Euler method was shown as follows.

$$y_{n+1} = y_n + hf(x_n, y_n) \tag{4}$$

where $y_{n+1}$ was the function value of the next point. $y_n$ was the function value of the current point. $h$ was the step size. $x_n$ was the *n*-th value point of the independent variable $x$. Based on the above process, a BQS model was constructed and solved to analyze the BQS process.

## Biofilm quorum sensing model based on ODE-PDE

Using ODE to analyze BQS provided a preliminary understanding of the process of bacterial BQS and simplified the core reactions of BQS. However, in practical analysis, it was necessary to analyze the quorum sensing of biofilms in complex situations. The PDE model could analyze multiple independent variables, describe spatial heterogeneity, and better analyze BQS. Based on the established ODE model, this study further analyzed the BQS process using PDE. The PDE model consisted of two parts including the control equation and the constraint conditions. The control equation consisted of unknown functions and their partial derivatives, physical parameters, and operators. The unknown function referred to temperature, displacement, and other physical quantities to be solved, and its partial derivative was an expression used to describe the rate of change of the independent variable of the unknown function. Physical parameters referred to constants obtained through experimental measurements or theoretical deductions that could reflect the characteristics of a system. Operators were mathematical operators that connected unknown functions and their partial derivatives. Constraint conditions referred to providing additional information to ensure that the solution of the equation was unique, physically reasonable, and conformed to the boundary or initial state of the actual problem. This constraint condition was mainly divided into boundary conditions and initial conditions. The boundary conditions were defined on the boundary of the solution area, which restricted the behavior of the solution on the boundary and was defined according to the actual problem. The initial condition was defined at the initial moment,

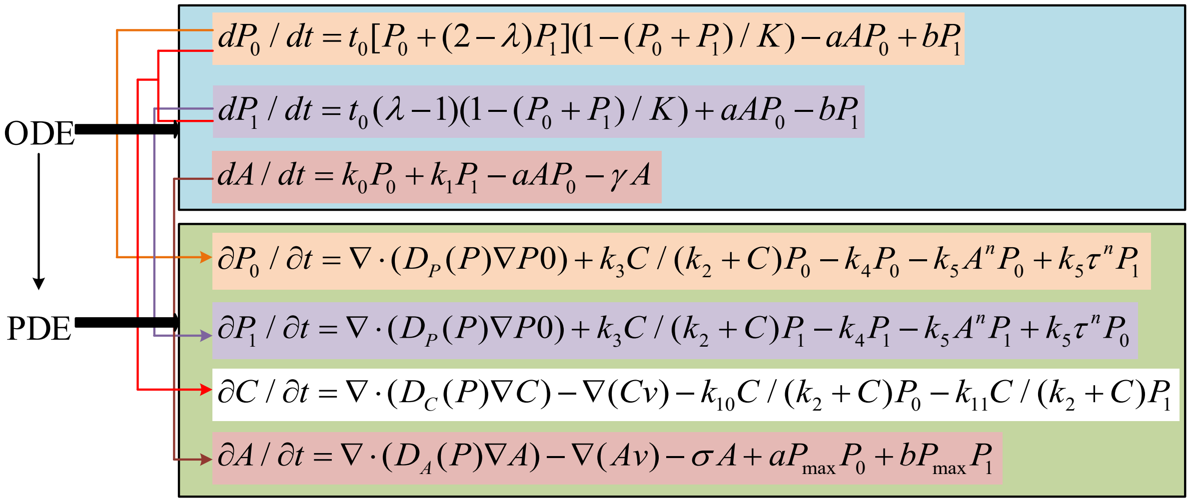


**Figure 3.** ODE-PDE biofilm quorum sensing model.

providing the starting point for the time evolution problem of the model. The complete mathematical expression of an object was defined by controlling equations and constraints, which were then solved. When modeling BQS using PDE, the core variables were first defined, and then the PDE function equations for the biofilm bacterial growth module and BQS signal molecules were constructed again based on the laws of physical conservation and mass conservation (Figure 2). In the biofilm bacterial growth process module, the function of decreasing biomass concentration in bacterial biomass concentration was shown below.

$$\partial P_0 / \partial t = \nabla \cdot (D_P(P)\nabla P0) + k_3C/(k_2+C)P_0 - k_4P_0 - k_5A^nP_0 + k_5\tau^nP_1 \quad (5)$$

where $P$ was the total concentration of bacterial biomass in the BQS process. $\tau$ was the threshold of quorum sensing signal molecules. $C$ was the concentration of nutrients in BQS. $k_3$ was the growth rate of bacteria per unit mass per hour. $k_2$ was the substrate concentration at which the bacterial growth rate reached half of its maximum value. $k_4$ was the rate of biomass death during the process. $k_5$ was the proportion of decrease in quorum sensing signal molecule activity or biofilm formation ability. $n$ was the number of repetitions of signal molecules in the process. The function of biomass concentration change in bacterial biomass concentration upregulation was shown below.

$$\partial P_1 / \partial t = \nabla \cdot (D_P(P)\nabla P0) + k_3C/(k_2+C)P_1 - k_4P_1 - k_5A^nP_1 + k_5\tau^nP_0 \quad (6)$$

The concentration function equation of nutrient $C$ was shown as follows.

$$\partial C / \partial t = \nabla \cdot (D_C(P)\nabla C) - \nabla(Cv) - k_{10}C/(k_2+C)P_0 - k_{11}C/(k_2+C)P_1 \quad (7)$$

where $k_{10}$ and $k_{11}$ were the consumption coefficients of $C$ by down regulating and up regulating biomass in BQS bacteria, respectively. $v$ was the flow velocity of signal molecules in BQS. The PDE for analyzing the concentration of bacterial BQS signaling molecules was shown as follows.

$$\partial A / \partial t = \nabla \cdot (D_A(P)\nabla A) - \nabla(Av) - \sigma A + aP_{\max}P_0 + bP_{\max}P_1 \quad (8)$$

where $P_{max}$ was the maximum concentration of bacterial biomass during BQS. $\sigma$ was the extinction coefficient of signal molecules. The study first used ODE to construct the basic functions related to the BQS process and then used the PDE model to analyze the various indicators in the basic functions to more clearly display the influencing factors in the BQS process (Figure 3).

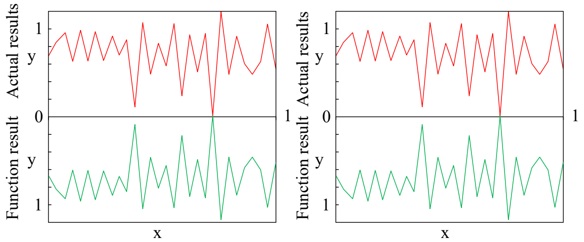
### Model test and statistical analysis

The performance of the proposed ODE-PDE model was tested on a computer equipped with a Linux (Ubuntu 20.04 LTS) operating system, 256 GB of memory, and an NVIDIA A100 80GB×2 hardware platform. The NumPy 1.26.0 (https://numpy.org/) was used as dependent library, and Python 3.10.12 (https://www.python.org/downloads/release/python-31012/) was employed as development tool. During the testing, the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) database (https://www.bv-brc.org/) was selected as the dataset for this study, which integrated a wealth of information related to biofilm structure, composition, and function. 500 data points each for *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, and *Staphylococcus aureus* were selected from the database. The diffusion coefficient of the ODE model was set to 1e$^{-4}$, while the initial reaction rate was set to 0.1, the spatial domain length was set to 1 m, the number of spatial grids was set to 100, and the time range was set to within 10 s. The subsequent comparisons were made between the ODE-PDE-based BQS analysis model and the widely used Optimized Biofilm Microbial Assembly (OBMA) analysis model (https://pmc.ncbi.nlm.nih.gov/articles/PMC10626739/), the quorum sensing system analysis model for *Vibrio harveyi* (VH) [19], and the fluid mosaic analysis model (FMM) [20]. Statistical analysis for the four models of proposed ODE-PDE, OBMA, VH, and FMM was conducted by using Python software (https://www.python.org). Each model was tested 50 times, and inter-group comparisons were performed by using ANOVA analysis with *P* vale less than 0.05 indicating statistically significant differences.

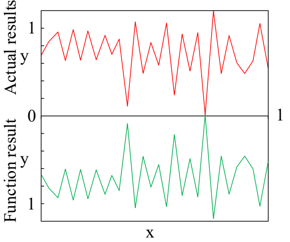
## Results

### Performance analysis of ODE-PDE model

The differences between the calculated results and the actual results were compared to reflect the prediction accuracy of the ODE-PDE model. The results demonstrated that the function representation obtained by the ODE-PDE model was almost identical to the actual results, indicating that the model could be used to accurately express the process of changes in biofilm structure (Figure 4).



(a) Biofilm structure (b) Biofilm composition



(c) Biofilm function

**Figure 4.** ODE-PDE model prediction accuracy.

The results of computation time and memory usage of the model showed that, when constructing the ODE-PDE model, the average memory consumption ratio of the computer was 32.2%. When the ODE-PDE model calculated class data, its average calculation time was 1.3 seconds. When using the ODE-PDE model to analyze biofilm composition and function data, the model had relatively low memory usage in the computer with average values of 31.7% and 32.1%, respectively. When calculating and analyzing data, its time consumption was also relatively low (Figure 5). The results confirmed that the ODE-PDE model had low computer memory usage and computation time when analyzing various data in biofilms, and most computers could meet this requirement. The ODE-PDE model was subjected to ablation experiments and the results showed that, when using structural data from biofilms to perform ablation experiments on the ODE-PDE model, the calculation accuracy of the ODE or PDE models was 86.3% and 82.6%, respectively. However, the calculation accuracy of the ODE-PDE model could reach 98.3%, which was much higher than that of

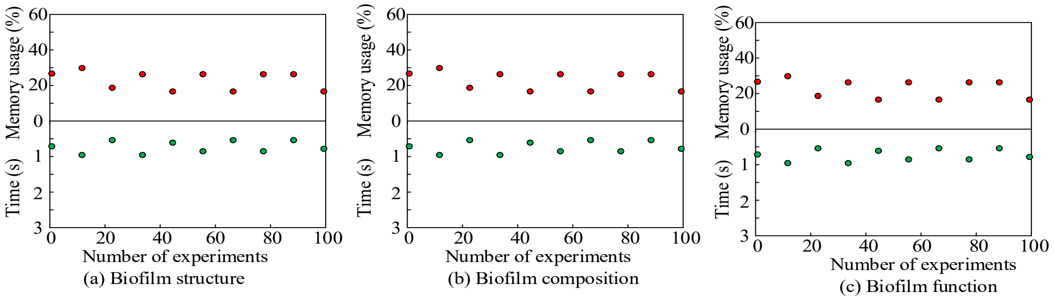


**Figure 5.** Analysis of model calculation time and memory usage.

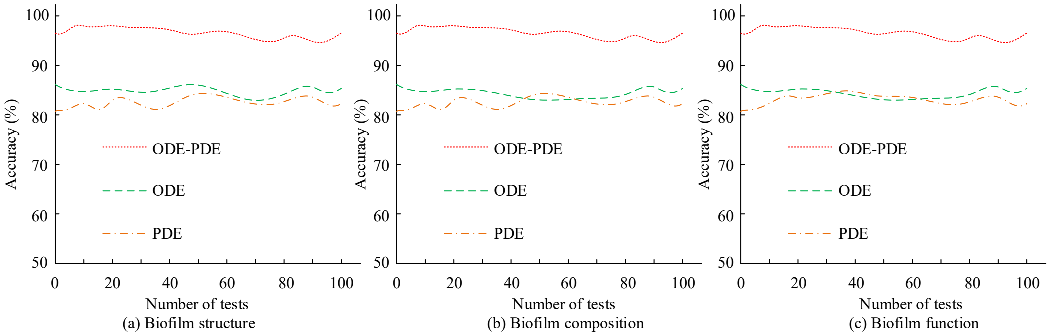
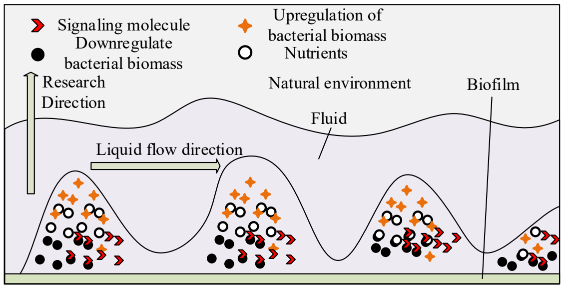


**Figure 6.** Results of ablation experiment.

the ODE or PDE models. Moreover, when validating the performance of the three models using biofilm composition data and structural data, the computational accuracy of the ODE model and PDE model in both datasets was below 90%, while the computational accuracy of the ODE-PDE model in both datasets averaged 98.4% and 98.6%, respectively (Figure 6). The results confirmed that the ODE-PDE model could improve its computational accuracy by combining the ODE and PDE models.

### Analysis of biofilm quorum sensing process

*Pseudomonas aeruginosa* was selected as the experimental object to analyze the quorum sensing process of its biofilm. The proposed ODE-PDE model was used to analyze the BQS process and construct an ODE-PDE model for the BQS process of *Pseudomonas aeruginosa*. The equivalent diagram of the mathematical model for quorum sensing of *Pseudomonas aeruginosa* biofilm included the flow of signal molecules, the upregulation and downregulation of bacterial biomass, and the flow of nutrients in the bacterial biofilm. The study investigated the vertical changes of four types of substances, which were the changes of various substances in the *Pseudomonas aeruginosa* biofilm during the quorum sensing process with the flow of liquid inside the bacteria (Figure 7).



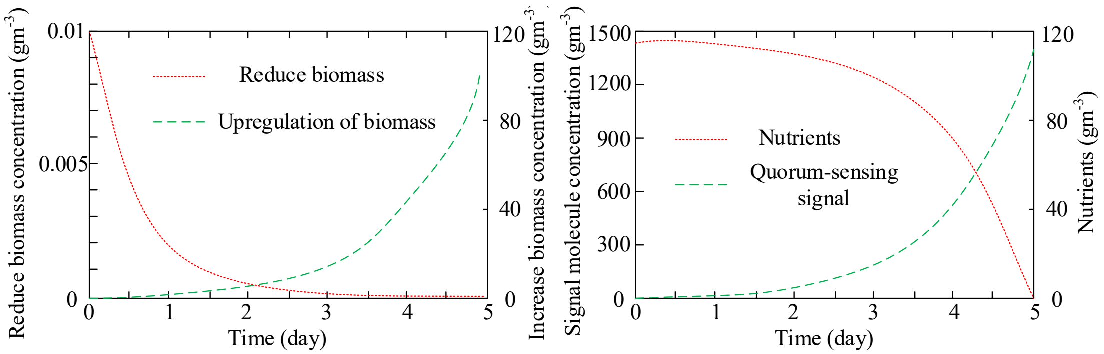
● $\partial P_0 / \partial t = \nabla \cdot (D_P(P) \nabla P0) + k_3 C / (k_2 + C) P_0 - k_4 P_0 - k_5 A^n P_0 + k_5 \tau^n P_1$

✦ $\partial P_1 / \partial t = \nabla \cdot (D_P(P) \nabla P0) + k_3 C / (k_2 + C) P_1 - k_4 P_1 - k_5 A^n P_1 + k_5 \tau^n P_0$

○ $\partial C / \partial t = \nabla \cdot (D_C(P) \nabla C) - \nabla (Cv) - k_{10} C / (k_2 + C) P_0 - k_{11} C / (k_2 + C) P_1$

❯ $\partial A / \partial t = \nabla \cdot (D_A(P) \nabla A) - \nabla (Av) - \sigma A + a P_{\max} P_0 + b P_{\max} P_1$

**Figure 7.** Equivalent diagram of mathematical model for quorum sensing of Pseudomonas aeruginosa biofilm.



(a) Changes in biomass concentration

(b) Changes in nutrient and quorum sensing signal concentrations

**Figure 8.** Concentration changes of various substances during biofilm quorum sensing process.

**Table 1.** Comparison of actual performance of analytical models.

| **Model** | **Analysis error** | | **Analysis time consumption** | |
|---|---|---|---|---|
| | **Error (%)** | ***P*** | **Time (s)** | ***P*** |
| ODE-PDE | 1.4±0.1 | - | 1.4±0.1 | - |
| OBMA | 5.7±1.2 | ** | 2.3±0.3 | * |
| VH | 9.3±1.7 | ** | 3.6±0.6 | ** |
| FMM | 12.6±4.3 | ** | 5.8±1.2 | ** |

**Note**: $^{*}$: $P < 0.05$. $^{**}$: $P < 0.01$.

The concentration changes of various substances in the bacterial biofilm during the quorum sensing process of *Pseudomonas aeruginosa* showed that, when using the ODE-PDE model to analyze the quorum sensing process of *Pseudomonas aeruginosa* biofilm with the change of time, the down regulated biomass concentration inside the bacterial biofilm was continuously decreasing and negatively correlated with time, while the up regulated biomass concentration was positively correlated with time and its concentration was continuously increasing. When the group sensing time was 3.5 days, the concentration of biomass was reduced to almost 0. Meanwhile, the increase in biomass concentration within the biofilm reached its maximum and continued to increase thereafter (Figure 8a). With the change of time, there was a negative correlation between nutrients and time in the process of BQS, while the concentration of quorum sensing signals was positively correlated with time (Figure 8b). The performance of the ODE-PDE was compared to OBMA, VH, and FMM models. The results showed that the error of the BQS concentration changes between the ODE-PDE model and the actual result was only 1.4 ± 0.1%, significantly lower than the errors of other models ($P < 0.01$). The analysis time of proposed model was also relatively low with an average of 1.4 ± 0.1 s, which was significantly lower than other models ($P < 0.05$) (Table 1). The proposed ODE-PDE model could accurately analyze the concentrations of various substances in the process of bacterial BQS. The analysis results helped relevant personnel coordinate the behavior of microbial communities, promote the common growth and reproduction of microorganisms, and promote the interaction between microbial communities and the environment.

## Discussion

To analyze the concentration changes of various substances in bacterial BQS process, an ODE-PDE model was proposed in this study. The Euler method was applied to address the ODE-PDE model construction and analyze the concentration changes of substances during quorum sensing. The performance of the ODE-PDE model demonstrated that the mathematical models of biofilm structure, composition, and function constructed by ODE-PDE had high similarity between the solved results and the actual results with the calculation accuracies reaching 98.3%, 98.4%, and 98.6%, respectively. The average time for solving the models was less than 1.5 seconds, which was similar to the results of Gulec *et al*. with the accuracy of 90.5% between the model solution results and the actual results, slightly lower than the proposed ODE-PDE model in this research [21]. It might be because they used the accelerated stochastic simulation method to analyze the changes in substance concentration in biofilms. However, their method could only analyze static quorum sensing processes and could not continuously analyze dynamically changing data during dynamic analysis, resulting in a large gap between its analysis results and actual results and low accuracy. The proposed ODE-PDE model in this research used ODE to analyze temporal variables and PDE that could ignore spatial heterogeneity to analyze the process of BQS in both time and space, thus achieving dynamic analysis. Therefore, the proposed ODE-PDE model had higher calculation accuracy. The bacterial BQS process of *Pseudomonas aeruginosa* was then tested by constructing the ODE-PDE mathematical model for the biofilm sensing process of this bacterium followed by analyzing the concentration changes of various substances during the bacterial biofilm sensing process. The results showed that the downregulation of biomass concentration and nutrients in the biofilm was negatively correlated with time, while the upregulation of biomass concentration and quorum sensing signal concentration was positively correlated with time. The results were roughly consistent with the research findings of Fang *et al*. [22]. In the BQS process of *Pseudomonas aeruginosa*, processes such as cell apoptosis and metabolism required the involvement of down regulated substances, and the degradation rate of down regulated biomass increased, leading to a decrease in the content of down regulated biomass. The growth and development of cell membranes required a large amount of nutrients, so the nutrients in bacterial biofilms were also decreasing. In the process of biofilm induction, bacteria promoted the synthesis of certain upregulated biomass through a series of gene expressions, resulting in an increase in the concentration of upregulated biomass by bacteria during this process. In addition, the induction of BQS by signal molecules was required to respond to the external environment and maintain the balance inside the biofilm, resulting in a continuous increase in its content. The ODE-PDE model proposed in the study could accurately analyze the process of BQS, thereby coordinating the process of BQS. However, in this study, only *Pseudomonas aeruginosa* was selected as the test object. Although statistical analysis was conducted, the research object was single. In the future, this model should be used to analyze various bacterial bioenergy quorum sensing models to further verify the performance of the model.

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