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**EXPERIMENTAL ARTICLES**

# Growth and Adhesion of *Pseudomonas fluorescens* in a Batch Culture: A Kinetic Analysis of the Action of Extracellular Antiadhesins

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**Abstract**—This work tests 6 hypothetical models simulating the growth, respiration, and adhesion of cells to the walls of the cultivation flask. All the models postulate the synthesis of antiadhesins (AAs), i.e., extracellular metabolites decreasing the degree of cell adhesion. The models have the following distinguishing features: (model 1) the blocking of sorption centers on the glass walls by antiadhesins (the competitive inhibition of adhesion); (model 2) the noncompetitive inhibition of adhesion; (model 3) the accelerated release of bound cells; (model 4) a combination of models 1 and 3; (model 5) a combination of models 1 and 3 with a delay; (model 6) a combined action of two AAs, one of which, $AA_1$, inhibits cell adhesion, and the other, $AA_2$ (its synthesis is induced when the concentration of $AA_1$ reaches a threshold level), stimulates the detachment of bound cells. Model 6 fits the relevant experimental data best. The delay effect is relatively small. The sigmoid character of the curve showing cell adhesion as a function of the antiadhesin concentration implies the existence of a strong cooperative effect in the adhesion inhibition. The models proposed satisfactorily simulate the growth, respiration, and adhesion of cells and AA synthesis in a batch bacterial culture grown either in a fresh nutrient medium or in the medium supplemented with the filtrate of a mature culture of the same species.

*Key words*: bacteria, reversible adhesion, *Pseudomonas fluorescens*, mathematical simulation.

The adhesion of microbial cells to solid surfaces is a vital adaptive strategy, which allows them to survive in diverse environments [1–3]. In the 1960–70s, the main effort of researchers was aimed at understanding the physicochemical regularities of microbial cell adhesion to the surface of various natural and artificial substances, such as soils, clays, glasses, ionites, and plastics [1–5]. Most of the mathematical models of cell adhesion were developed in terms of equilibrium thermodynamics (more specifically, in terms of the free energy of cells adsorbed to the surface of solid substrates) and van der Waals and electrostatic forces [1]. At present, however, it has been recognized that microbial adhesion cannot be adequately described in only physicochemical terms, since cell adhesion strongly depends on the physiological state of microorganisms, in particular, on the metabolites secreted into the medium and on the composition of the microbial cell wall [6–10]. The adhesion of living organisms is specific and biologically justified, occurs under metabolic control, and involves particular organelles, such as flagella and fimbriae.

In a series of our recent publications, we have described the dependence of pseudomonad adhesion on the presence of specific microbial exometabolites in the medium [11–13]. Some of these exometabolites (the so-called adhesins) are presumably lectin-like proteins with sticky ends, which reversibly (and regulatorily) bind microbial cells to the surface of glass, plastics, and other materials. Exometabolites of the opposite action (the so-called antiadhesins, AAs) are synthesized under favorable growth conditions to give a signal that microbial cells may detach from the solid surface and propagate over the whole volume of an accessible medium. The growth of microbial populations with allowance made for reversible cell adhesion and synthesis of extracellular adaptation factors is far from being well understood. In addition to direct experimental approaches (the identification of signal molecules, the obtaining of mutants defective in adhesion, the isolation and cloning of the respective genes, etc.), reversible cell adhesion can also be fruitfully studied by mathematical simulation, more specifically, by the comparative kinetic analysis of relevant experimental data and data obtained by mathematical simulation [14]. To the best of our knowledge, such an approach has been used for the investigation of culture growth or synthesis of microbial products [15], but never for the analysis of reversible cell adhesion.

The aim of the present work was to test several alternative hypotheses accounting for the growth and adhesion of bacterial cells controlled by extracellular antiadhesins with the use of mathematical simulation. Several hypothetical models developed by us were verified by comparing their predictions with the relevant experimental data.

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## DESCRIPTION OF PROPOSED MODELS

**The scope of the model.** The range of phenomena related to the adhesion of microbial cells to solid surfaces is sufficiently wide. Suffice to say that the phenomenon of cell adhesion is responsible for the formation of microbial communities in soil, subsoil, and aquatic (periphyton) microbial communities; cell attachment to tissues of higher organisms initiates most of infections and attachment to free surfaces starts any colonization process. It should be noted that cell adhesion *per se* is often complicated by more complex and irregular physicochemical and biological processes.

The present publication is an attempt to understand in depth the self-regulated process of cell adhesion, which involves the synthesis of specific cell metabolites, adhesins and antiadhesins. This aim can be achieved by analyzing growing (and, hence, physiologically normal) bacterial populations, the simplest of which is a homogeneous batch culture growing exponentially with a constant cell generation time. Specifically, we will consider a bacterial culture growing under the following conditions:

(1) the presence of sufficient amounts of glucose (a potentially limiting substrate) in a mineral synthetic growth medium and the absence of substrate inhibition (this was achieved by using glucose at initial concentrations not exceeding 2 g/l);

(2) the absence of an extended lag phase (this was achieved by using inocula that were adapted to the growth medium);

(3) the absence of product inhibition (this was achieved by using exponential-phase cultures grown for no more than 4–6 h);

(4) a constancy of physicochemical conditions (growth temperature, pH, ionic strength, and contact area), which is necessary to maintain a constant growth rate and to provide for the reproducibility of cell adhesion;

(5) an intense mixing of the cell suspension, which prevents the formation of concentration gradients of substrates and products and exerts uniform shear force on attached cells.

**The notion of reversible adhesion.** Under the aforementioned presumptions, cells in a batch culture growing in a shaken flask adhere to the flask walls under constant physicochemical conditions (pH, ionic strength, ionic composition, temperature, and contact area). The only parameters that change are the concentrations of cells and their metabolites. The intense mixing of the culture, its population homogeneity, and the constancy of physicochemical conditions provide for the reversibility and reproducibility of cell adhesion, i.e., for such characteristics that are typical of the adhesion of individual chemical compounds. The reversibility of cell adhesion implies the achievement of steady-state, when the opposite processes of cell binding and cell release exactly compensate each other:

$$\text{free cells } (x) \underset{\text{desorption } k_2}{\overset{\text{adhesion } k_1}{\rightleftharpoons}} \text{bound cells. } (y)$$

The equilibrium is established provided that sorption depends on the concentration of free cells ($x$), whereas desorption depends on the concentration of adsorbed cells. In the simplest case, when these dependences are linear, the dynamic equilibrium is achieved at $k_1x = k_2y$. Cell adhesion is considered to be irreversible, if adhesion bonds cannot be ruptured mechanically, for instance, by flask shaking, active cell motion, or convective cell transfer. In such a case, $k_1x \gg k_2y$, and the adsorbed cells begin to form a biofilm. Strong adhesive interactions are typical of nonhomogeneous conditions (e.g., in the absence of mixing), when cells are enclosed in the glycocalyx, a viscous extracellular high-molecular-weight polysaccharide-containing material produced by starving microorganisms. The production of the glycocalyx may last weeks or even months. This process will not be considered here, as its simulation requires complex distributed mathematical models accounting for the spatial inhomogeneity of the system and the population heterogeneity of cells. In the case of a homogeneous batch culture, the possible irreversibility of cell adhesion can be neglected, at least for time periods no longer than several hours. The reversibility of cell adhesion in our experiments follows from the fact that the intense shaking of the cultivation flask led to cell detachment from the flask walls into the medium. Furthermore, the microscopic examination of glasses immersed in a bacterial culture revealed the dynamics of reversible cell sorption similar to that of the optical density of the culture ([11] and unpublished data).

Guided by the principle "First learn to walk, then run," we deliberately leave the problem of irreversible cell adhesion, which is by no means widely spread in nature and is much more important in human practice than reversible cell adhesion [1–5, 7, 10], for our future investigations and publications.

**Description of the basic model.** The basic mathematical model (Table 1) is a system of three ordinary differential equations, which describe the dynamics of (1) free unbound cells in the liquid phase of a culture; (2) cells attached to the walls of the cultivation flask; and (3) antiadhesin (AA) molecules in the medium. For simplicity, the area of the active surface involved in cell adhesion was expressed in an implicit form, and the concentration of both kinds of cells (free and bound) were measured in the same units (mg dry biomass/l culture liquid). It was also assumed that the culture volume and the surface area of the sorbent (the cultivation flask walls), as well as the conditions of cultivation and cell interaction with the flask walls, did not change in the course of experiments.

**Table 1.** The basic model simulating the growth and adhesion of microorganisms in a batch culture

| Variable | Equation |
|---|---|
| Concentration of free cells, $x$ | Growth – Adhesion + Detachment<br>$\frac{dx}{dt} = \mu x - k_1 x(e_0 - y) + k_2 y$ (1) |
| Concentration of bound cells, $y$ | Growth + Adhesion – Detachment<br>$\frac{dy}{dt} = \mu y + k_1 x(e_0 - y) - k_2 y$ (2) |
| Concentration of AA in the medium, $m$ | Biosynthesis – Degradation<br>$\frac{dm}{dt} = \Theta\mu(x + y) - k_3 m$ (3) |

Note: $\mu$ is the specific growth rate expressed in $h^{-1}$ (a constant); $e_0$ is the cell-binding capacity of the incubation flask walls expressed in mg/l (a variable); $k_1$ is the second-order kinetic constant characterizing the cell attachment rate to the flask walls, expressed in l/(h mg); $k_2$ is the first-order kinetic constant characterizing the cell detachment rate from the flask walls into the liquid phase, expressed in $h^{-1}$; $k_3$ is the first-order kinetic constant characterizing the spontaneous degradation rate of AA, expressed in $h^{-1}$; $\Theta$ is a dimensionless coefficient characterizing the amount of a substrate consumed for the synthesis of AA.

The mathematical formalism of cell adhesion logically follows from the very notion of its reversibility (see above). The binding of cells to glass was presented as the second-order reaction of their interaction with the sorption active centers (ACs) on the free glass surface. The cell adhesion rate was taken to be proportional to the cell concentration in the liquid phase ($x$) and the difference ($e_0 - y$). The reverse process of cell detachment from glass was described in terms of the first-order reaction, whose velocity was proportional to the number of bound cells. The constant $k_2$ of this reaction can be interpreted as the probability of the transition of a bound cell into the culture liquid calculated per unit time. It was also assumed that binding to a solid surface did not affect the metabolic activity of cells and that the growth rate of free and bound cells were the same. The latter assumption agrees with the relevant experimental data [11, 17]; however, it is not of crucial importance, since equations (1) and (2) will otherwise contain two independent variables accounting for the growth rate of two groups of bacterial cells. All cells were assumed to be able to adhere to the glass surface, the adherence being suppressed or completely inhibited by antiadhesins [11]. In other words, we assumed a population homogeneity of the simulated culture. If the culture is not homogeneous, its heterogeneity can be taken into account by dividing the population of cells into several groups: the group of AA producers, the group of cells receiving signals, etc. Alternatively, cells can be distinguished on the basis of the levels of their growth rate and AA synthesis, by specifying subsets of $x$ and $y$ variables, introducing continuous distribution functions, and solving equations in partial derivatives.

AA is known to be synthesized constitutively in proportion to the cell biomass. Bacterial growth under altered conditions (either unfavorable or favorable) causes respective changes in the rate of AA synthesis, either decreasing or enhancing it. When analyzing various models, we determined which kinds of cells (bound or unbound) are more active with respect to the synthesis of antiadhesins.

**The mechanism of antiadhesin action on the reversible sorption of cells.** The hypotheses proposed (Table 2) are based on the principles of enzymatic kinetics. Competitive inhibition in the process of cell binding (hypothesis 1) implies that antiadhesins and lectin-like compounds bind to the same active centers (AC) located on the cell surface. The alternative hypothesis that ACs are located on the glass surface was rejected by a simple experiment, in which the flask was rinsed with the culture liquid filtrate before cell inoculation. The competitive mechanism of inhibition suggests a decrease in the number of available ACs, designated $e_0$. Equations (4), (5), and (7) were written by analogy with the respective equations of enzymatic kinetics, parameter $K_i$ being the dissociation constant of the complex of antiadhesin with the active center. Parameter $n$, which determines the sigmoid dependence of the cell adsorption inhibition on the inhibitor concentration, can be interpreted as a measure of cooperativity in the reversible interaction of AA with AC: the larger $n$, the greater the increase in the affinity of AA toward AC after the binding of the first molecules of AA to lectin-like compounds [16].

Noncompetitive inhibition (hypothesis 2) implies that the targets of lectins and antiadhesin are different and that the kinetic binding constant $k_1$ decreases by virtue of AA effect on adhesive properties of lectins.

Hypothesis 3 postulates that antiadhesin does not diminish the cell binding rate but facilitates the detachment of adsorbed cells from the flask wall. This fact is taken into account in the respective second-order equa-

**Table 2.** Formalization of alternative hypotheses for the regulation of cell adhesion to the flask walls

| Action of AA | Supplement to the basic model | |
|---|---|---|
| | model element | equation |
| Competitive inhibition of cell adhesion | Cell-binding capacity ($e_0$) | $e_0 = \frac{E}{1 + m^n/K_i}$ (4) |
| Non-competitive inhibition of cell adhesion | Cell adhesion rate ($k_1$) | $k_1 = \frac{K_1}{1 + m^n/K_i}$ (5) |
| Activation of cell desorption | Cell detachment rate into the liquid phase ($k_2$) | $k_2 = \eta m^n$ (6) |
| Mixed competitive inhibition and activation | Cell-binding capacity ($e_0$) and cell detachment rate ($k_2$) | $e_0 = \frac{E}{1 + m^n/K_1}$ (7) |
| | | $k_2 = \eta m^n$ (7a) |
| Inhibition and activation with a delay | Intracellular concentration of AA ($p$) | $\frac{dp}{dt} = \Psi(m - p)$ (8) |
| | | $k_2 = \begin{cases} \eta p^n, \text{ if } y > y^* \\ 0, \quad \text{if } y \le y^* \end{cases}$ (8a) |
| $AA_1$ ($m_1$, [5]) suppresses cell adhesion, $AA_2$ ($m_2$) stimulates cell detachment, synthesis of $AA_2$ is initiated when $m^*$ reaches a threshold level | Synthesis of $AA_2$ ($m_2$) and cell detachment rate ($k_2$) | $\frac{dm_2}{dt} = \begin{cases} \Theta' m y - k_4 m_2, \text{ if } m \ge m^* \\ 0, \quad \text{if } m < m^* \end{cases}$ (9) |
| | | $k_2 = \eta m_2^n$ (9a) |

tion, according to which the rate of cell detachment is proportional to the number of adsorbed cells and the concentration of AA in the medium.

Hypothetical model 4 postulates a combination of the competitive inhibition of cell binding and the stimulation of the detachment of bound cells.

The principle of biological inertia in hypothesis 5 implies that a metabolite (in the given case, AA) exerts its physiological action (in the given case, the release of glass-attached cells) after a lag phase, during which the concentration of this or another metabolite reaches a threshold level (in the given case, $y^*$).

Best-fitting hypothesis 6 postulates the existence of two types of antiadhesins, $AA_1$ and $AA_2$. $AA_1$ inhibits cell adhesion by diminishing the rate of cell adherence to the glass surface, whereas $AA_2$ stimulates the release of glass-attached cells. It is $AA_2$ that is subject to biological inertia: its synthesis begins only when the concentration of $AA_1$ in the medium reaches a threshold value. It should be noted that model 6 is applicable to a wide range of phenomena, including those which are poorly understood at a molecular level. The wide applicability of model 6 is related to its specific features, such as the existence of thresholds, which is typical of the biological processes of transition from one state to another, and the multiplicity of the adhesion-inhibiting factors, which include antiadhesins, lectin-hydrolyzing enzymes, the change of the living form, the sloughing of the extracellular sheath, etc.

## EXPERIMENTAL TESTING OF THE MODELS

**Simulation technique.** Numerical integration and other calculations were carried out using the Model Maker 2 software package (SB Technology Ltd.), which allows original and inverse problems to be solved, i.e., (1) to derive curves showing the dynamics of all of the primary and derivative model components based on the specified values of model parameters (or coefficients) and (2) to choose the best-fitting sets of parameters minimizing the residuals between calculated and experimental data. Experimental data were obtained from our previous publications [11, 12] and from the publications of other authors [7, 8].

**Growth and adhesion dynamics in a batch culture without adding CL.** Figure 1 shows the dynamics of cells in a batch culture of *P. fluorescens* [7]. Measuring the culture density with great accuracy and at sufficiently short intervals made it possible to observe the so-called adhesion trough, i.e., a decrease in the cell density within 0.5 to 1.5 h after inoculation, caused by cell adhesion to the cultivation flask walls. The subsequent steep rise in the cell density was due to the super-

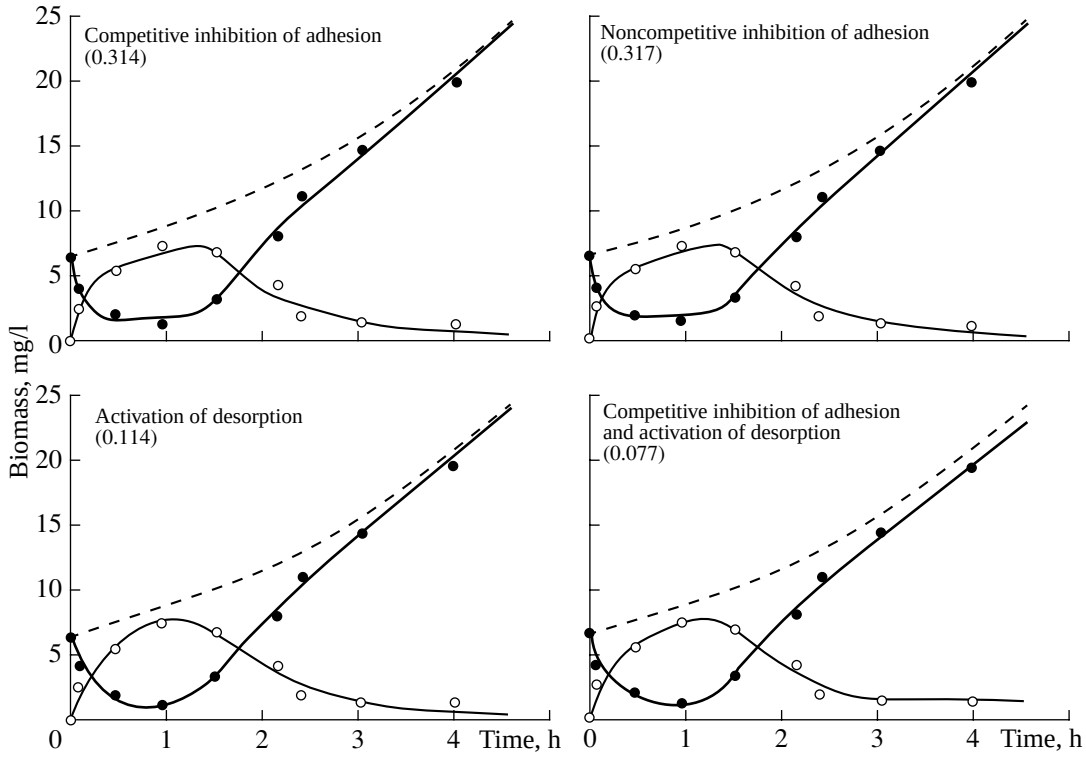


**Fig. 1.** Simulation of the growth and adhesion of bacterial cells by four different mathematical models. Experimental data are for a batch *P. fluorescens* culture (taken from [7]). Data for free and bound cells are shown by black and open circles, respectively. The total cell biomass is shown by dotted lines. The curves were calculated according to the equations in Tables 1 and 2. Curves for models 4 and 5 are not shown, as they do not differ from the curves calculated by model 3. Parenthesized are the relative errors of simulations. The optimized values of model parameters are as follows:

| Parameter | Hypothesis 1 | Hypothesis 2 | Hypothesis 3 | Hypothesis 6 |
|---|---|---|---|---|
| μ | 0.29 | 0.29 | 0.29 | 0.29 |
| $e_0$ | 20 | 20 | 20 | 20 |
| Θ | 0.2 | 0.2 | 0.2 | 0.2 |
| $n$ | 44 | 25 | 3.1 | 2.3 |
| $k_1$ | 0.336 | 0.337 ($K_1$) | 0.184 | 0.191 |
| $k_2$ | 1.30 | 1.31 | 6.40 (η) | 0.88 (η) |
| $k_3$ | 0 | 0 | 0 | 0 |
| $k_4$ | – | – | – | 1.36 |
| $K_i$ | $1.4 \times 10^6$ | $10.3 \times 10^6$ | $3.5 \times 10^8$ | $9.06 \times 10^3$ |
| $M$* | – | – | – | 0.33 |
| Θ' | – | – | – | 1.0 |

position of the exponential growth of free cells and the desorption of glass-attached cells into the medium. After 3–4 h of incubation, the cell density followed an exponential curve, whose extrapolation to the time zero gave an inoculum value. This fact made it possible to estimate the amount of cells adsorbed to the glass walls from the difference between the current concentration of free cells and the extrapolated exponential growth curve. The degree of agreement between the dynamic curves simulated in terms of the 6 models proposed (Table 2) and the experimental growth curves was estimated from the relative residuals between experimental and calculated data, which were determined by the formula: $(x_{exp} - x_{calc})/x_{calc}$, where $x_{exp}$ and $x_{calc}$ are the experimental and calculated values of the cell biomass, respectively.

A comparison of the six models showed that hypotheses 1 and 2, postulating, respectively, the competitive and noncompetitive inhibition of cell sorption, showed the least agreement with the respective experi-

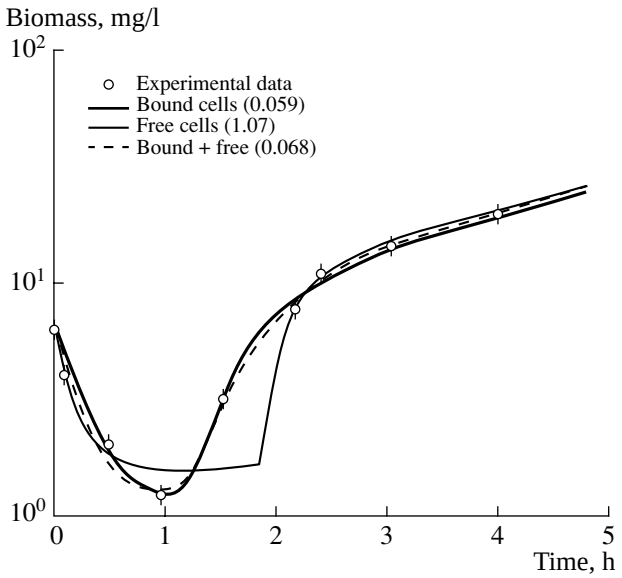


**Fig. 2.** Simulation of the effect of antiadhesins on the adhesion of bacterial cells by model 6 using equations (3a) and (3b). Antiadhesins were considered to be synthesized either by free cells, or by bound cells, or by both. The vertical bars on the experimental curves show the confidence interval (0.95). Parenthesized are the squares of relative residuals. The best results are obtained under the assumption that antiadhesins are only synthesized by bound cells (the relative residual square is 0.059).

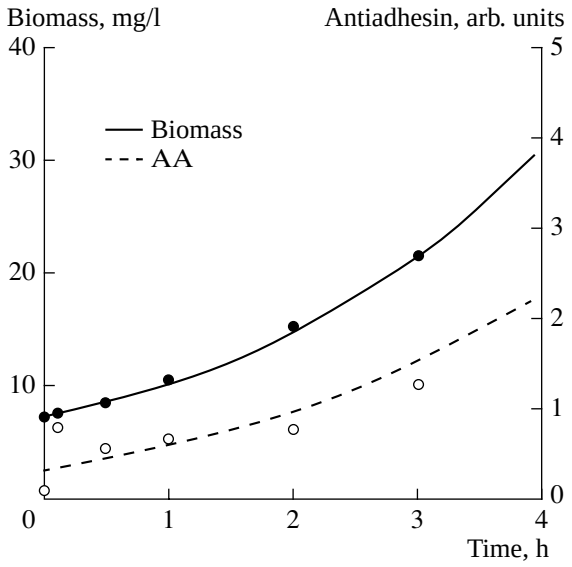


**Fig. 3.** Simulation of the growth of *P. fluorescens* and the accumulation of antiadhesins in the medium. Experimental data are from [8]. AA were assayed by testing the culture liquid. The curves were calculated in terms of model 1.

mental data: the calculated curve describing culture density either underestimated the adhesion maximum or overestimated cell adhesion at the very beginning of the dynamic curve. Allowance for the stimulation of cell desorption under the action of antiadhesin (hypothesis 3) considerably improved the agreement between experimental and calculated data. The combination of the competitive inhibition of cell binding and the stimulation of cell detachment in model 4 and the account for biological inertia in model 5 insignificantly improved the adequacy of the simulation.

Model 6, which postulates the existence of two types of antiadhesins, $AA_1$ (inhibits cell adhesion) and $AA_2$ (stimulates the release of glass-attached cells), turned out to be the best-fitting model. A numerical simulation showed that the relative residuals between experimental and calculated data was minimal (0.059) under the assumption that antiadhesins are only synthesized by glass-attached cells (Fig. 2). This made it possible to simplify the equation (3) of the basic model by eliminating the variable *x*.

The basic model accurately simulated the dynamics of antiadhesin synthesis (Fig. 3), indicating the validity of the assumption that AA is synthesized constitutively in proportion to the other main cellular components. The absence of the adhesion trough on the growth curve presented in this figure can be accounted for by an elevated initial concentration of AA in this particular experiment.

**The effect of exogenously added antiadhesins on cell adhesion.** The existence of extracellular antiadhesins is directly confirmed by the finding that cell adhesion in a culture can be suppressed by adding the filtrate of another culture in which cell adhesion is absent. The basic model in all 6 variants agrees with the experimental dependence of cell adhesion on the concentration of the AA well (Fig. 4 exemplifies this with reference to model 1), except for the shape of the dynamic curve.

It should be noted that the addition of the culture liquid filtrate diminished the magnitude of the adhesion trough but did not affect the position of the local minimum in the concentration of free cells, *x* (Fig. 5). In other words, antiadhesins present in the culture liquid filtrate decrease the degree of cell adhesion without influencing its position in time. None of the modifications of the basic model, except for model 6, satisfactorily described the position and shape of the adhesion trough. For instance, model 3 predicted a shift of the local minimum of *x* toward lower *t* with the increasing concentrations of added antiadhesins (Fig. 5c), which contradicts experimental data (Fig. 5a). At the same time, predictions in terms of model 6 showed good agreement with the respective experimental data. This was achieved by introducing two conditions. First, the synthesis of $AA_2$ (the antiadhesin that stimulates the release of glass-attached cells) was supposed to begin when the concentration of $AA_1$ reached a threshold level $m^*$ (0.33 arb. unit, in comparison with the maximum value of this parameter equal to 1.2 arb. unit). Second, $AA_2$ was supposed to be an unstable compound (either volatile, or insoluble, or labile during filtration), since experimental data indicated that the culture liquid filtrate added to the tested culture contained only $AA_1$

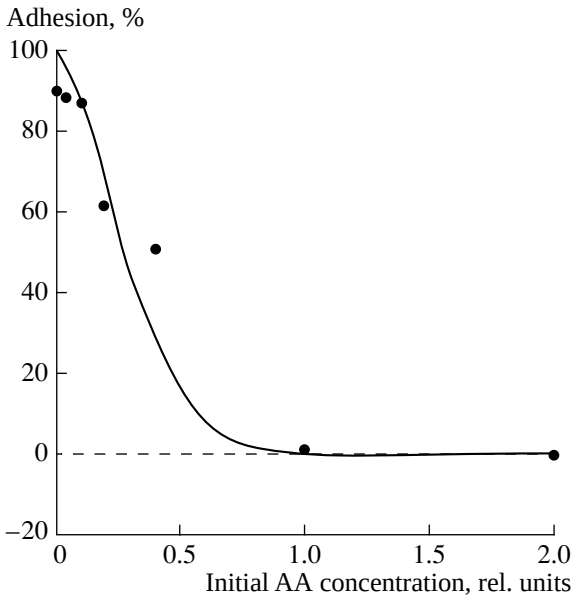


**Fig. 4.** The effect of exogenously added antiadhesins on the adhesion of *P. fluorescens* cells to the walls of the cultivation glass flask. Experimental data are from [8]. AA were added in the form of the cell-free filtrate. Cell adhesion was evaluated in relative units, taking the degree of cell adhesion at the time of adhesion trough as 100%. The curves were calculated by model 1.

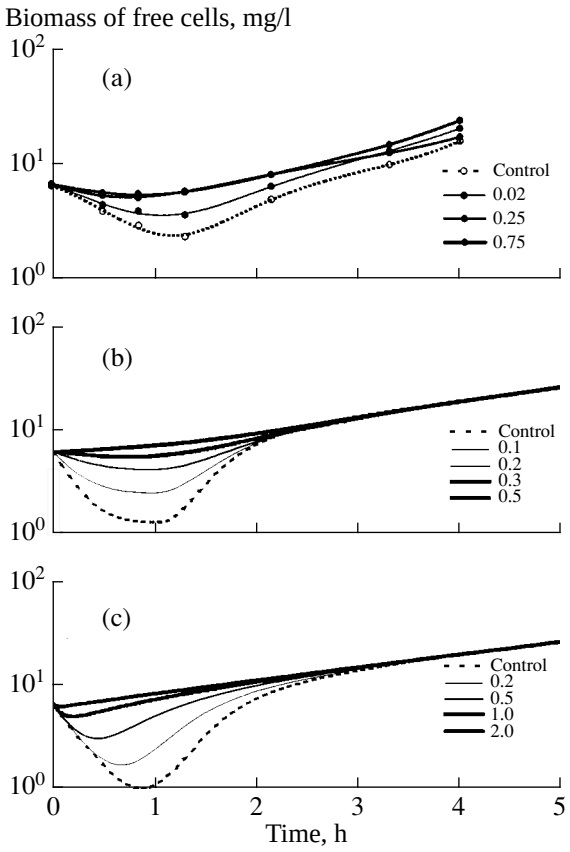


**Fig. 5.** The effect of the amount of antiadhesins added at the time of inoculation on the biomass of free cells in a batch culture: (a) experimental curves; (b) curves calculated by model 6; and (c) curves calculated by model 3. AA doses shown in the figure panels are in relative units.

(an inhibitor of cell adhesion). The occurrence of some discrepancy between experimental data and those predicted by model 6 suggests that the culture liquid filtrates contain some factors, other than AA, which affect the dynamics of bacterial growth. The effect of these factors can be taken into account by more complex models of bacterial growth.

**Bacterial growth and respiration.** The mathematical models of cell adhesion were verified with respect to not only cell density but also cell respiration. It was taken into account that the total respiration of a culture is due to the respiratory activity of both free and adsorbed cells. The cell respiration rate $v$ was calculated as :

$$v = Y_{p/x}(x + y) + v_{\text{waste}}, \qquad (10)$$

where $Y_{p/x}$ is the $CO_2$ yield per biomass unit (equal to 0.625 g $CO_2$/g biomass), and $v_{\text{waste}}$ is futile respiration not related to bacterial growth (see [15] for details).

Due to the high degree of cell adhesion (because of a large area of the glass surface) in the experiment with the parallel measurements of the cell biomass and respiration [17], the cell density $x$ decreased throughout the incubation period. This fact, together with the occurrence of a significant lag phase, compelled us to supplement the basic model with the following equation of the physiological state $r$ [15]:

$$\frac{dr}{dt} = \mu_m r(1 - r) \qquad (11)$$

with the substitution of $\mu_m r$ for $\mu$ in equations (1)–(3) and (10).[1]

The new basic model in all six versions (Table 2) showed satisfactory agreement with experimental data (Fig. 6). The drop in the density of free cells was accompanied by an exponential rise in the respiratory activity of cells. This fact reflects an almost exponential growth of the total biomass of free and glass-attached cells.

The addition of exogenous antiadhesins present in the culture liquid filtrate stimulated not only the release of adsorbed cells but also the respiratory activity of the culture. To fit experimental data, two initial conditions of the basic model, i.e., the concentration of antiadhesins and the parameter $r_0$, were changed. The necessity of changing the first parameter is evident, since the

[1] The variable $r$ is a measure of the relative content of nonconstitutive cellular components (such as RNA, ribosomes, and enzymes of primary metabolism) necessary for acceleration of the growth of microorganisms. Unlike these components, secondary metabolites provide resistance to unfavorable environmental conditions. The $r$ value varies from 0 to 1.0. If $r_0$ (the value of $r$ for the inoculum) is less than unity, there is a lag phase, during which the specific growth rate $\mu$ gradually increases to reach a value of $\mu_m$.

added CL filtrate raised the concentration of antiadhesins in the medium. The necessity of increasing the parameter $r_0$ from 0.02 to 0.06 indicates that the CL filtrate probably contain not only AA but also some growth-promoting factors. They may represent either growth factors of specific hormone-like metabolites. Their investigation will be the subject of our further simulation studies.

## DISCUSSION

The kinetic analysis of the growth, respiration, and reversible sorption of *P. fluorescens* cells provided deeper insight into processes occurring in a batch culture. The best-fitting model postulates the existence of two types of antiadhesins, $AA_1$ and $AA_2$. $AA_1$ inhibits cell adhesion by decreasing the rate of cell adhesion to the glass surface, whereas $AA_2$ stimulates the release of glass-attached cells. $AA_1$ is probably synthesized by adsorbed cells in amounts proportional to the total cellular synthesis. The synthesis of unstable $AA_2$ begins only when the concentration of the other antiadhesin ($AA_1$) reaches a threshold level.

The effect of delay or biological inertia is relatively weak. The sigmoid character of the curve describing cell adhesion as a function of the antiadhesin concentration is indicative of the existence of a strong cooperativity effect. This implies that the effect of low AA concentrations is insignificant, but at increasing AA concentrations, the effect of each of the AA molecules steeply rises. The nature of this phenomenon is unclear and calls for further investigation.

The models proposed satisfactorily describe the growth, respiration, and adhesion of cells and AA synthesis in a batch bacterial culture grown in a fresh nutrient medium or in the medium supplemented with the filtrate of a mature batch culture. The models quantitatively describe the effect of the CL filtrate, including the dependence of the degree of cell binding and the independence of the local minimum in the concentration of free cells on the amount of the added filtrate.

The inferences made from the simulation studies are in agreement with the data obtained experimentally. In particular, we showed that the antiadhesin $AA_1$, inhibiting cell adhesion, is a mixture of *n*-alkanes [18] and that the antiadhesin $AA_2$, promoting the desorption of attached cells, is a proteolytic enzyme that is able to digest adhesin. The synthesis of adhesin (which is a protein or glycoprotein) and several antiadhesins, as well as the related sensory system of signal transduction, requires high energy expenditures. Nevertheless, cells incur these energy losses, since regulated cell adhesion play a significant role in the survival of bacterial populations. It is tempting to speculate that when occurring in a new environment, pseudomonads (and likely other opportunistic bacteria) first attach to a solid surface, since the probability of surviving under unfavorable conditions is higher in a bound than in a free state. Indeed, the concentration of nutrients is higher at the interface between the liquid and solid phases, which is of particular importance in the case of nutrient deficiency. The adsorption of bacterial cells facilitates transport processes between them, reduces energy expenses for the maintenance of bacterial populations, and diminishes risks associated with predators and the effects of various stresses (thermal, osmotic, radiation, etc.). However, when the environmental conditions become favorable, the attached state is no longer beneficial for bacterial cells. Then exometabolites of the antiadhesin-type may serve as information signal of retreat: “Do not worry! Environment is favorable, detach and move around!” In other words, antiadhesins may play the role of a uniform indicator of favorable environments.

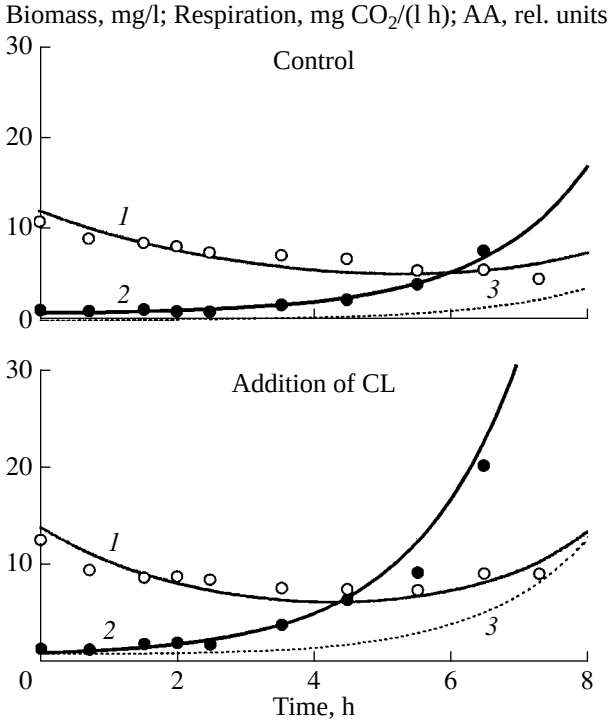


**Fig. 6.** Simulation of the dynamics of (*1*) free cells, (*2*) cell respiration, and (*3*) the concentration of antiadhesin in the medium. Experimental data are from [11]. The upper panel shows data for a batch *P. fluorescens* culture without adding the culture filtrate. The lower panel shows data for the same culture supplemented with the culture filtrate. The curves were calculated by model 1.

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