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STUDY OF A PHENOMENOLOGICAL MODEL OF THE KINETICS OF BACTERIAL ADSORPTION ON LOW-ENERGY SURFACES

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The mathematical model of the kinetics of bacterial adsorption on polymeric materials with different hydrophobicities proposed earlier was analyzed. The calibration procedure and the algorithm of calculations for the simulation model were described. The model was used for describing the experimental data on the adsorption of *E. coli* (strain 055) and *Listeria monocytogenes* bacteria on various polymeric surfaces. The theoretical forecast of the experimental results indicated that the accuracy of the forecast decreased with the lowering of the wetting angle on the surface of the polymer.

In our previous paper [1] we obtained the basic differential equations of the model for the kinetics of bacterial adsorption. The main problem solved in the first part was the issue of resolution of the motive forces of adsorption in relation to the nature of their origin. The present work concerns the calibration procedure and the theoretical predictions based on this model. In this connection another important problem arises: the problem of comparing the results of different adsorption experiments based on the so-called adhesion protocol covering the experimental conditions of obtaining the data in question. Unfortunately, different authors use dissimilar adhesion protocols. The model developed earlier [1] removes this uncertainty to some extent by employing a specially introduced parameter.

CALIBRATION OF THE MODEL

Experimental Data for the Calibration of the Model

The experimental data used in the calibration of the model were taken from an earlier publication [2]. In this experiment bacteria *Escherichia coli* (strain 055) were grown on agar within 24 h at 37°C. *Listeria monocytogenes* microorganisms were cultured on a soy-containing medium within 48 h at 37°C. Further on, the bacteria were precipitated, triply washed in Hank's saline solution by repeated centrifuging, and finally reprecipitated in a liquid medium with specified surface tension (the latter value was varied by changing the concentration of DMSO in Hank's saline solution [2]).

After that, 1 ml of a bacterial suspension containing 10^8 bacteria in a corresponding medium was applied to the surfaces. The bacteria were incubated within 30 min at $T = 21°C$; subsequently, the surfaces were washed with Hank's medium to remove the bacteria that were not adhered to the surface. After drying the samples, we counted the number of cells adsorbed on the surface. Information on the wetting angles of bacterial and adsorbing surfaces used in the experiments is presented in Tables 1 and 2.

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Table 1

Experimentally Determined Values of the Wetting Angles for Water on Polymers (γ_{LV} = 72.0 mN/m)

Polymer		
Material	Wetting angle of water (°)	Surface tension γ_{SV} (mN/m)
Fluorinated copolymer of ethylene and propylene	110 ± 3	16.7 ± 1.7
Polystyrene	95 ± 2	25.6 ± 1.2
Low density polyethylene	84 ± 4	32.5 ± 2.5
Acetal resin	64 ± 1	44.8 ± 0.6
Sulfonated polystyrene	24 ± 3	66.4 ± 1.3

Note. The values of the surface tensions were calculated using the equation of state.

Table 2

Experimentally Determined Values of the Wetting Angles on Bacteria in Hank's Saline Solution (γ_{LV} = 72.8 mN/m)

Microorganisms		
Strain	Wetting angle of the saline solution (°)	Surface tension γ_{BV} (mN/m)
E. coli 055	16.7 ± 1.0	69.9 ± 0.3
L. monocytogenes	26.1 ± 1.2	66.3 ± 0.5

Note. The values of the surface tensions were calculated using the equation of state.

THE METHOD OF CALCULATION

The calculations were run on an IBM PC (Pentium 200 processor). The computer program was written in Fortran-90. The input parameters were: the value of the wetting angle of the liquid on the surface of the bacterial cell, the value of the wetting angle of the medium on the polymer surface, the surface tension of the medium, the density of the cells in the medium, and the average projected area of the bacterial cell. The output parameter was the number of cells adsorbed on the unit surface within a specified time interval. The trapezoid method [3] was used for the numerical integration of differential equations.

The calculations were run in accordance with the algorithm stated in items I_A–I_D:

I_A: calculation of γ_{SV} based on the experimental values of γ_{LV}, $\cos\Theta_{SL}$ (Table 1) using Eqs. (5) and (6);

I_B: calculation of γ_{BV} based on the experimental values of γ_{LV}, $\cos\Theta_{BL}$ (Table 1) using Eqs. (7) and (8);

I_C: calculation of γ_{BV} based on the above-calculated values of γ_{BV} and γ_{BV} using a similar set of equations inferred from the Young equation and the equation of state;

I_{D1}: input of the current value of γ_{BV}; I_{D2}: calculation of $\gamma_{LV}\cos\Theta_{SL}$ using γ_{LV} and γ_{LV}; I_{D3}: calculation of $\gamma_{LV}\cos\Theta_{SL}$ using γ_{LV} and γ_{LV}; I_{D4}: calculation of ΔG_h using γ_{LV}, γ_{LV}, and γ_{LV}; I_{D5}: numerical integration of differential Eqs. (1) and (3); I_{D6}: return to Step I_{D1}.

When analyzing different media for the specified solid surface—bacterial type combination, items from I_A to I_C are executed once whereas items from I_{D1} to I_{D6} are iterated for each value of the surface tension of the medium in question.

IDENTIFICATION OF THE PARAMETERS OF THE MODEL

The model was calibrated on a portion of the data obtained in the foregoing experiment. Used in this experiments were bacteria with the wetting angle not exceeding 30° and in concentration $N_{ext} = 10^{14}$ 1/m^3. The ϕ_1, ϕ_2, and Ψ values in Eqs. (1) and (2) [1] were identified for *L. monocytogenes* and a fluorinated ethylene–propylene copolymer (Fig. 1*a*). The identification procedure based on the least squares method gave in this case the following values: $\phi_1 = 6 \times 10^{-8}$ (m/s), $\phi_2 = 0.205$ (m/mN), $\Psi_{monocyt} = 1.05$. The accuracy of identification was above 95%. For the *E. coli* culture and for the same solid material the only parameter to be identified was $\Psi_{E.\ coli}$. In the $\Delta G_h > 0$ area this parameter was equal to 0.56. For this case the numerical values of ϕ_1 and ϕ_2 were taken to be equal to the values obtained for the *L. monocytogenes* culture. Here we made use of the fact that according to our approach the values of wetting angles and surface tensions determine unequivocally the rate of adsorption caused by hydrophobic interactions, regardless of the nature of the interacting surfaces.

Figure 1*a* presents the results of the identification procedure and the corresponding experimental data. It should be noted that the mechanism governing the density of bacteria on the surface is of a stochastic nature. Hence, the area of projection of the bacterial cell (S^{BAC}) is a random value.

In our calculations we assumed that the value of this parameter is 1.44×10^{-12} m^2. This value correlates with the typical geometrical dimensions of bacteria. In order to simplify the comparison of our results with the experimental data, we present the $N(t)$ function as the number of bacteria per 10^4 mm^2 in all graphs.

RESULTS AND DISCUSSION

In order to utilize the developed model, it is needed to understand the structure of the $\Psi_{monocyt}$ and $\Psi_{E.\ coli}$ parameters because no theoretical assumptions about them were made above. These functions were used as constants in the calculation procedure. An analysis of experimental data will make it possible to draw certain inferences. The experimental data characterizing the adsorption of *E. coli* (Fig. 1*a*) contain the region of the surface tension of the liquid (64–69 mN/m) where $\Delta G_h > 0$. Inside this region the value of ΔG_h changes considerably. Nevertheless, the number of adsorbed cells per unit area inside this interval does not depend on the value of the surface tension of the liquid. If the repulsion within this region has existed, the number of adsorbed cells should have decreased. However, this suggestion is not confirmed by the experiment. This fact was generalized and used for the determination of the $\Psi_{monocyt}$ and $\Psi_{E.\ coli}$ values within the $\Delta G_h > 0$ region. It is also seen from the experimental results that the number of bacteria adsorbed on unit area within the region of lower values of the surface tension of the liquid does not very strongly depend on the type of the adsorbing material. Based on this fact, we assumed that these results are determined by the type of bacteria.

Taking the foregoing assumptions into account, we used the model for describing the remaining portion of the experimental data. The determination of the ϕ_1, ϕ_2, $\Psi_{monocyt}$, and $\Psi_{E.\ coli}$ constants in the calibration procedure for one type of material allowed us to predict all remaining types of materials using the same constants. Figure 1*b–e* displays the theoretical and experimental curves. It can be seen from Fig. 1 that the accuracy of the prediction of the experimental results decreases with a decline in the wetting angle of the polymer surface. Nevertheless, the theoretical prediction gave acceptable results for the number of bacteria adsorbed on unit area for materials with the wetting angle not exceeding 60°. The curves in Fig. 1*e* demonstrate the lack of coincidence between the model and the experiment. Therefore, in the situation where the adhesion of hydrophilic microorganisms to hydrophilic surfaces is considered, our approach does not give the required degree of accuracy. This can be explained by the inapplicability of the equation of state to hydrophilic surfaces.

Figure 2*a* shows the theoretical prediction of the adsorption kinetics for *L. monocytogenes*. The calculations were performed for several types of the adsorbing materials at the constant value of the surface tension of the liquid (72 mN/m) on the assumption that the wetting angle may be stable for a long period of time. All curves in Fig. 2*a* tend to the limiting value representing the situation of a complete filling of the surface. The curves in Fig. 2*b* show the dynamics of the effective wetting angle of the polymer surface in time expressed by Eq. (9). The curves in Fig. 2*b*, as well as those in Fig. 2*a*, tend to the limiting values, which are the wetting angle of the surface of the bacterial wall. It should be noted that the existence of this limiting value is the consequence of bacterial repulsion, which is postulated in this work.

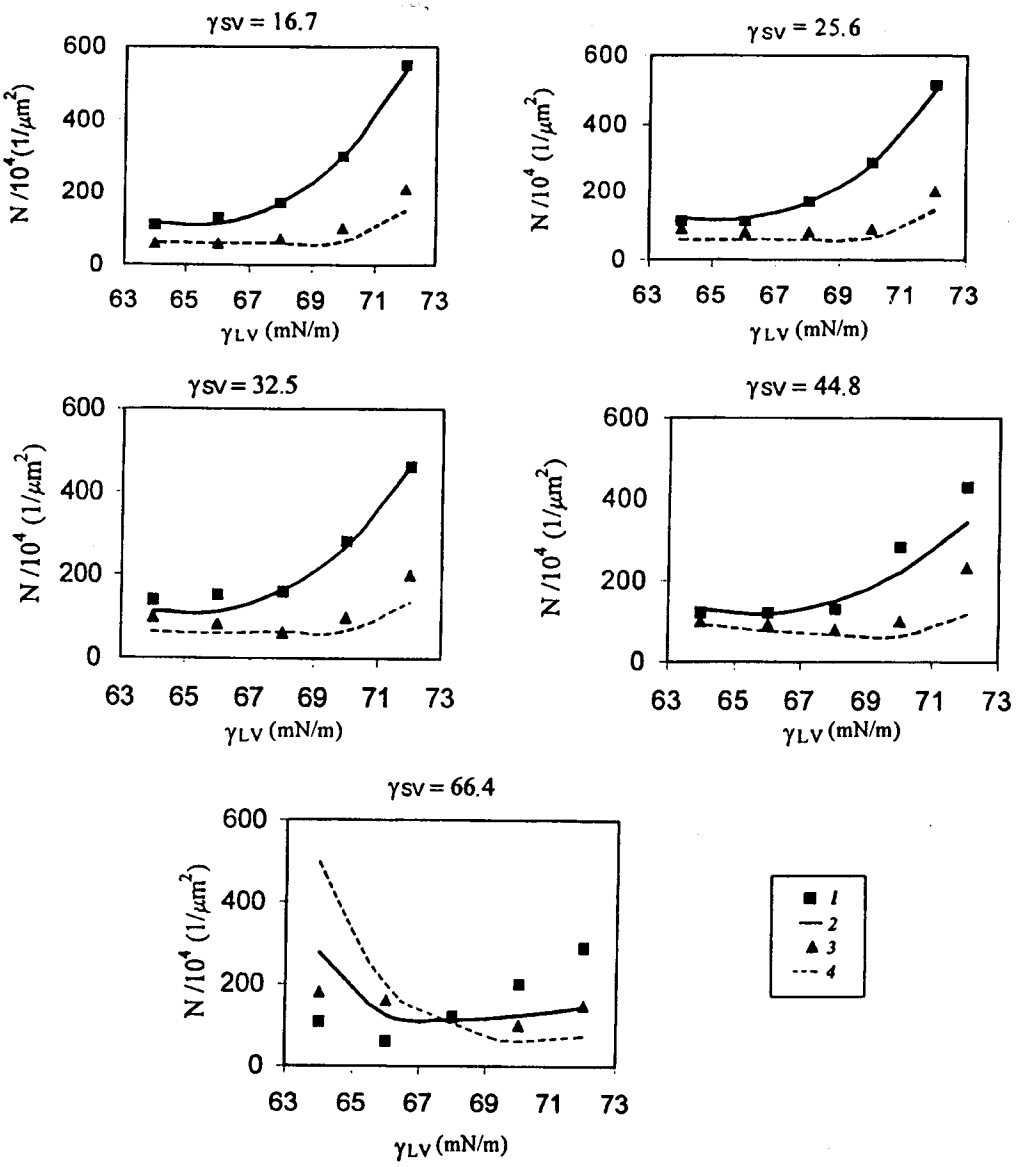

Fig. 1

Number of bacteria adsorbed on unit area within 30 min as a function of the surface tension of the liquid (the concentration of cells in the surroundings was 10^8 ml^{-1}); *a*: fluorinated copolymer of ethylene and propylene; *b*: polystyrene; *c*: low-density polyethylene; *d*: acetal resin; *e*: sulfonated polystyrene. *1*: *L. monocytogenes*, the experiment; *2*: *L. monocytogenes*, the model; *3*: *E. coli* 055, the experiment; *4*: *E. coli* 055, the model.

Based on the results of our study, the following conclusions can be made. The model developed is capable of describing the rate of bacterial adsorption for bacteria with the wetting angle not exceeding 30° and for surfaces with the wetting angle not lower than 60° with an acceptable accuracy. The accuracy of the prediction of the number of bacteria adsorbed on unit area per unit time decreases with the lowering of the wetting angle of the adsorbing surface. The existence of repulsion between bacteria gives rise to the limiting value of the number of the bacteria adsorbed on unit area. The first term in Eq. (1) characterizing the hydrophobic part of the kinetics is similar to the Arrhenius law for the kinetics of the first-order chemical reaction.

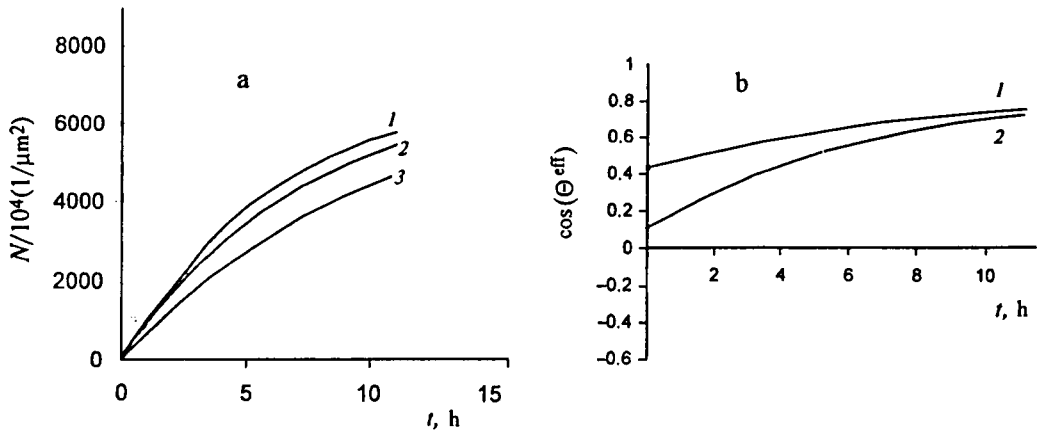

Fig. 2

Results of simulation of the process of adsorption of *L. monocytogenes* cells (γ_{BV} = 66.3 mN/m) from the aqueous phase (γ_{LV} = 72 mN/m) for different types of the adsorbing materials: the dynamics of the bacterial accumulation as a function of time (*a*), the dynamics of the effective wetting angle as a function of time (*b*).

ADDENDUM

Notation

Ψ is the value of the contribution of nonhydrophobic interactions to the adsorption process (dimensionless), BL is the bacterial cell–liquid interface, BS is the bacterial cell–polymer interface, BV is the bacterial cell–water vapor interface, ΔG_h is the hydrophobic portion of the free energy of adhesion (mN/m), $N(t)$ is the number of bacteria adsorbed on unit area ($1/m^2$), N_{ext} is the number of bacteria in the surroundings ($1/m^3$), SL is the polymer–liquid interface, SV is the polymer–water vapor interface, S^{access} is the surface area accessible for bacteria (m^2), S^{tot} is the area of the material participating in the adhesion process (m^2), S^{bac} is the projection area of the bacterial cell (m^2), γ_{LV} is the surface tension of the liquid (mN/m), γ_{BL} is the interphase tension in the bacterial cell–liquid system (mN/m), γ_{SL} is the interphase tension in the polymer–liquid system (mN/m), γ_{BS} is the interphase tension in the bacterial cell–polymer system (mN/m), γ_{SV} is the interphase tension in the polymer–water vapor system (mN/m), γ_{BV} is the interphase tension in the bacterial cell–water vapor system (mN/m), ϕ_1 is a constant (m/s), ϕ_2 is a constant (m/mN), Θ_{BL} is the wetting angle of the liquid on the surface of the bacterial cell (°), Θ_{SL} is the wetting angle of the liquid on the surface of the polymer (°), Θ_{BS} is the effective wetting angle of the liquid on the surface of the polymer partly occupied by the bacteria (°).

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