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EXPERIMENTAL ARTICLES

A Study of the Adsorption of Bacterial Cells on Porous Materials

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Abstract—The paper presents experimental data on the adsorption of bacterial cells on porous materials.

Key words: adsorption, immobilization, bacterial cells, porous adsorbents.

Microbiological methods are widely used in industry. The immobilization of microorganisms on properly chosen adsorbents stimulates microbial metabolism, protects cells from unfavorable agents, and preserves their physiological activity [1, 2].

The immobilization of microbial cells on various materials can be chemical (due to the presence of reactive groups, such as $–NH_2$, –OH, –COOH, or –SH, on the cell surface) [3], electrostatic (in electric fields) [4], mechanical (when an attached microbial cell is not able to move by itself but is accessible to substrates) [1, 5], or physical (adsorption) [2]. The immobilization of microbial cells by adsorption is a simple, affordable, and universal method of their preservation in a physiologically active state [7].

The adsorption (adhesion) of microorganisms is similar to the adsorption of colloid particles. The linear size of microbial cells (1–10 μm) promotes their adhesion [6]. There are many factors (such as the age and the physiological state of cells) that influence the sorption of microbial cells. The surface structures of bacterial cells (flagella and other appendages) [5, 8], as well as hydration effects (which are due to the hydrophilicity/hydrophobicity balance between the cells and the adsorbent) [1, 8], also play an important part in the cell adherence to solid surfaces. The composition of the medium, its pH, and environmental conditions considerably influence the adsorption of cells by changing their electrokinetic potential [8]. The surface properties of adsorbents also affect the process of cell immobilization. Good adsorbents have a specific area of more than 0.01 m^2/g [5]. However, the degree of cell immobilization also depends on the structure and the size of adsorbent pores [9] and may not be proportional to the specific area [1, 5, 7]. For the maximum adsorption of dividing microbial cells, the adsorbent pores must be 2–5 times greater than the cells. For the maximum adsorption of budding microbial cells, the pore diameter must exceed the cell size by 4 times. Of interest is the fact that spore-forming microorganisms are adsorbed most when the pore size either coincides with the spore size or exceeds it by about 4 times [1, 5].

The nature of adsorbents is also important. Organic adsorbents are chemically stable and show a great variety of surface properties and pore structures, whereas inorganic adsorbents are resistant to biological degradation, are affordable, and can be easily regenerated [5]. The disadvantage of inorganic adsorbents is that they are soluble in alkaline solutions.

Adsorbents for cell immobilization are typically chosen empirically since theoretical approaches to this are not as yet developed. There are several major groups of adsorbents: natural inorganic materials (bentonite, kaolinite, cordierite [10], zeolite, diatomite, kieselguhr, sands, silicates, carbonates, phosphates, perlite, sponge [7]); natural organic materials (chitin, chitosan, dextran, wood, bagasse, collagen, silk, wool, lignin [5]); inorganic and carbon-containing artificial materials (silica, silica gel [11], glass, graphite, carbon black, charcoal [12], fabrics, fibers, brick, ceramics, magnetite, oxides, hydroxides [9, 17], synthetic polymers, and combined materials [5].

The aim of this work was to determine such parameters according to which optimal adsorbents for the immobilization of bacterial cells can be chosen.

MATERIALS AND METHODS

Experiments were carried out with the hydrocarbon-oxidizing bacteria *Bacillus mucilaginosus* [13] and *Acinetobacter* sp. [14].

The organic and inorganic, natural and artificial, materials used for immobilization are listed in the table.

The sorption characteristics of porous materials were determined by routine methods [15]. The specific area (S_{sp}) of adsorbents was determined by the method of the thermal desorption of argon. The saturation volume of the sorption space (*Ws*) was determined by

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Relevant parameters of the porous adsorbents under study

Adsorbent	Ws, cm^3/g		S_{sp}, m^2/g	V_Σ	V_{ma}	S_{ma}, m^2/g	R_{pore}, nm	Surface charge
	with H_2O	with C_6H_6		cm^3/g				
Carbon-containing and organic materials								
AG-PR coal	0.26	0.34	900	0.95	0.55	0.22	5500	–
Coal coke	0.04	0.03	0.3	0.05	0.02	0.05	1000	–
Petroleum coke	0.05	0.04	0.03	0.98	0.93	0.39	5000	+
Sansorb	0.24	0.21	2	1.22	0.83	2.10	800	–
Shungisite	0.001	0.16	0.4	0.31	0.10	0.05	50	–
Peat	0.16	0.04	0.03	0.32	0.13	0.05	500	–
Inorganic materials								
Expanded clay	0.01	0.06	0.04	0.64	0.54	1.35	800	–
Porous concrete	0.12	0.18	28	0.32	0.20	1.95	25	+
Perlite	0.05	0.003	1	3.21	2.90	7.20	800	–
Vermiculite	0.07	0.06	2.2	0.92	0.81	0.36	500	–
Fired clay	0.02	0.10	14	0.25	0.15	0.07	6000	–
Lava	0.004	0.004	0.3	0.01	0.01	0.08	200	–
KSKG	0.11	0.71	250	1.10	0.65	0.43	15	–
Bentonite	0.15	0.18	30	0.86			4000	+

the desiccator method either with water or benzene vapors at $P/P_S = 0.95$ (to avoid the formation of liquid on the surface of adsorbents). The total volume of pores (V_Σ) was calculated as the difference of the reversals of the apparent and pycnometric densities of materials. The volume, the specific area, and the predominant radius of macropores were determined by the method of mercury porometry.

The titer of viable cells immobilized on 1 g of adsorbent (T_c) was calculated as the difference of the titers of the cell suspension before and after immobilization. The number of cells in a suspension was determined by the Koch method [16]. *Acinetobacter* sp. and *B. mucilaginosus* cells were in contact with adsorbents for 4 and 20 h, respectively. Cells for experiments were taken from the exponential growth phase.

RESULTS AND DISCUSSION

The dependence of the cell titers T_c on the major parameters of the porous structure of adsorbents was presented in the most descriptive manner, i.e., graphically.

As is evident from Figs. 1 and 2, the integral parameters of porous adsorbents (the specific area and the total volume of pores) did not show values that would be optimal for the efficient adsorption of microbial cells. This can be explained by the different contributions of pores with different sizes (macro-, meso-, and micropores) to the adsorption of cells on porous adsorbents.

It is known that only macropores whose linear size is within 0.1–30 μm can contribute to the immobilization of microbial cells [15]. However, the analysis of the number of adsorbed cells as a function of the volume (Fig. 3) and the specific area (Fig. 4) of such macropores also did not show a clear dependence of these parameters. Indeed, even at the minimal volume

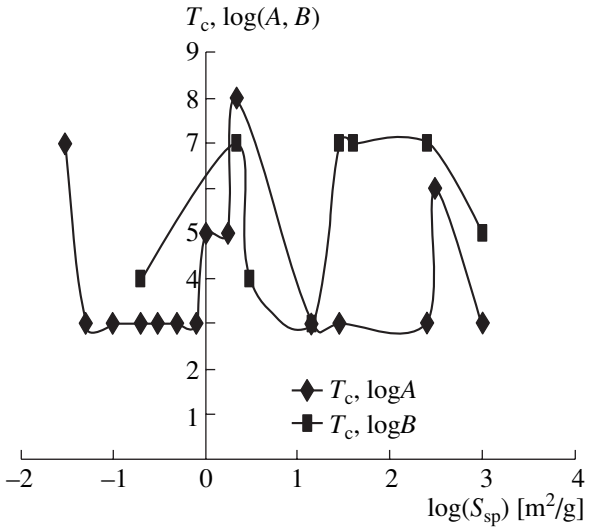

Fig. 1. The effect of the specific area of adsorbents on the titer of adsorbed *Acinetobacter* sp. (log$A$) and *B. mucilaginosus* (log$B$) cells.

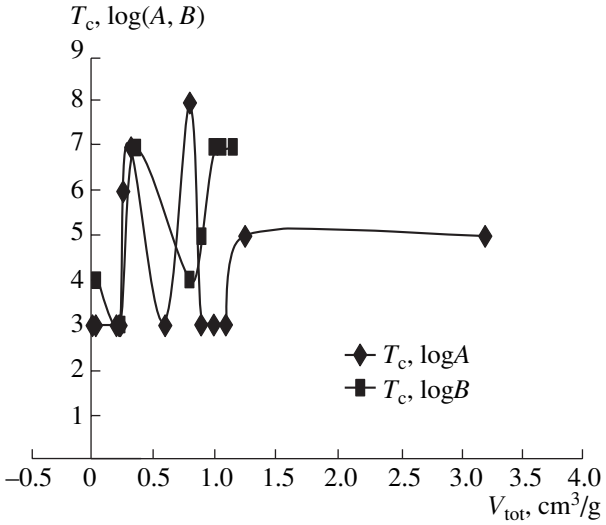

Fig. 2. The titer of adsorbed *Acinetobacter* sp. (log*A*) and *B. mucilaginosus* (log*B*) cells versus the total volume of adsorbent pores.

of macropores (0.01 cm^3/g), the cell adsorption was at a maximum. Simple mathematical calculations make it possible to estimate the minimum volume of macropores that is optimal for the immobilization of microorganisms. At the macropore radius $r_{ma} = 3$ μm (the minimum radius of the macropores that can accommodate the microbial cells studied), the volume of one macropore is $V_1 = 4/3 \times \pi \times r_{ma}^3 = 1.13 \times 10^{-10}$ cm^3. At $T_c = 10^7$ cells/g, the adsorbing capacity of porous materials is sufficiently high. Consequently, the minimum volume of macropores required for the efficient adsorption of microbial cells is $V_{ma}\text{min} = V_1 \times T_c = 1.13 \times 10^{-3}$ cm^3/g, i.e., ≈0.001 cm^3/g.

For cylindrical pores, their surface and volume are related as $S = 2V/r$. In the given case, $S_{ma}\text{min} = 2V_{ma}\text{min}/r_{ma} = 7.53 \times 10^{-4}$ m^2/g, i.e., ≈0.001 m^2/g.

The results of these calculations do not contradict our experimental data or the data available in the literature and show that microbial cells can be efficiently immobilized on porous materials with a volume of macropores of about 0.001 m^2/g, provided that the macropores are sufficiently large to accommodate the microbial cells and that the chemical structure of the adsorbents promotes cell attachment.

The dependence of the number of adsorbed cells on the surface hydrophilicity of adsorbents is shown in Fig. 5. The hydrophilicity was estimated from the saturation volume of the sorption space determined with water vapor *Ws* (H_2O). The latter parameter of ten adsorbent samples was below 0.07 g/g, and, consequently, these samples were rather hydrophobic. Three adsorbent samples showed *Ws* (H_2O) values higher than 0.24 g/g (i.e., they were rather hydrophilic). The cell titers T_c for both hydrophobic and hydrophilic adsorbents were minimal. At the same time, 9 of the 11 adsorbents with *Ws* (H_2O) values between 0.07 and 0.24 g/g exhibited the maximum value of T_c, i.e., the maximum cell adsorption. This can be explained by the specific interaction of the hydrated surfaces of adsorbents and cells, as is evident from the absence of a similar relationship between the number of immobilized cells and the saturation volume of the sorption space determined with benzene vapor *Ws* (C_6H_6) (Fig. 6). The involvement of hydration effects in cell adsorption can be explained as follows: In aqueous media, cells are hydrated. If adsorbents are hydrophobic (*Ws* (H_2O) < 0.07 g/g), they repel hydrated cells, preventing their immobilization. This explains the weak adsorption of microbial cells on shungisite, expanded and fired clays, lava, perlite, and coke. On the other hand, the very high

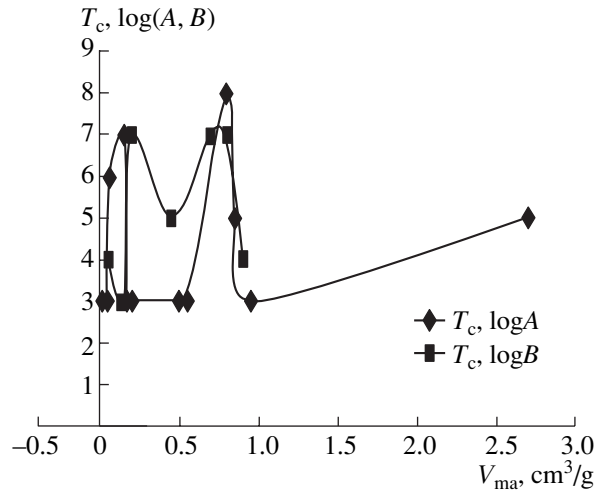

Fig. 3. The titer of adsorbed *Acinetobacter* sp. (log*A*) and *B. mucilaginosus* (log*B*) cells versus the volume of adsorbent macropores.

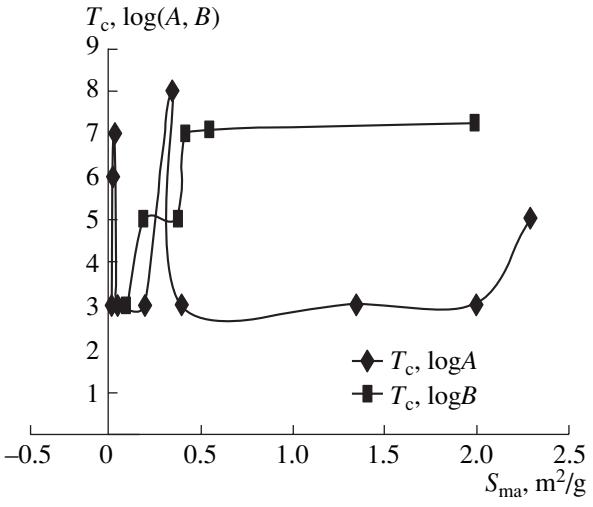

Fig. 4. The titer of adsorbed *Acinetobacter* sp. (log*A*) and *B. mucilaginosus* (log*B*) cells versus the specific area of adsorbent macropores.

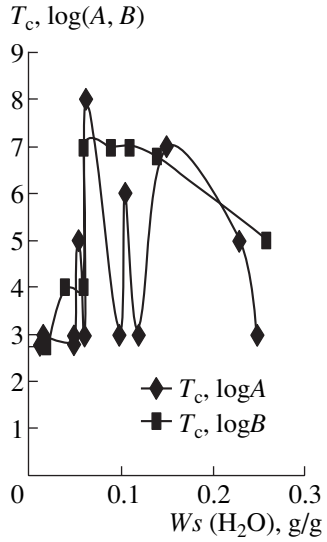

Fig. 5. The dependence of the titer of adsorbed *Acinetobacter* sp. (log$A$) and *B. mucilaginosus* (log$B$) cells on the saturation volume of the sorption space determined with water vapor.

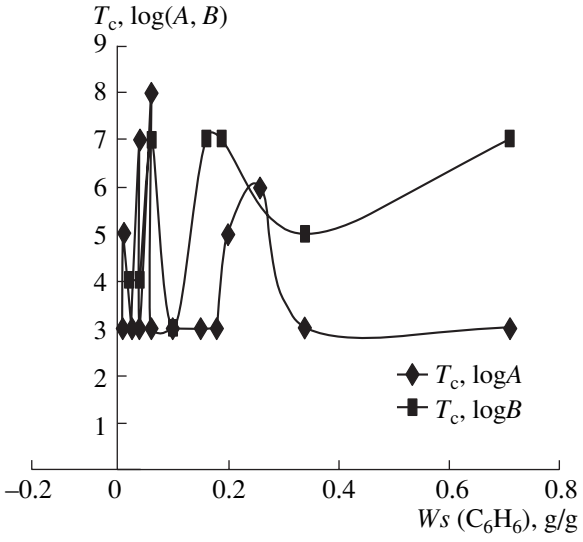

Fig. 6. The dependence of the titer of adsorbed *Acinetobacter* sp. (log$A$) and *B. mucilaginosus* (log$B$) cells on the saturation volume of the sorption space determined with benzene vapor.

hydration of the adsorbent surface (Ws (H_2O) > 0.24 g/g) also impedes the adsorption of hydrated cells [17]. This is the case with sansorb and AG-PR coal. Other parameters of adsorbents and cells may also affect cell immobilization, due to which some materials show atypical sorption. For instance, porous concrete and silica gel readily adsorb *B. mucilaginosus* cells but poorly adsorb *Acinetobacter* sp. cells, both of which are hydrophilic. In this case, cell adsorption is likely to be influenced by such factors as the surface charge of cells and adsorbents and the proportion between the cell and pore sizes. The slime-producing *B. mucilaginosus* cells tend to adsorb on positively charged surfaces since these cells have a negatively charged surfaces and their slime is hydrophilic. For this reason, *B. mucilaginosus* cells readily adsorb on the positively charged surfaces of bentonite, porous concrete, and petroleum coke, whereas *Acinetobacter* sp. cells readily adsorb on negatively charged surfaces.

The dependence of cell adsorption on the predominant radius of adsorbent pores is shown in Fig. 7. It is known that *Acinetobacter* sp. cells have a size of

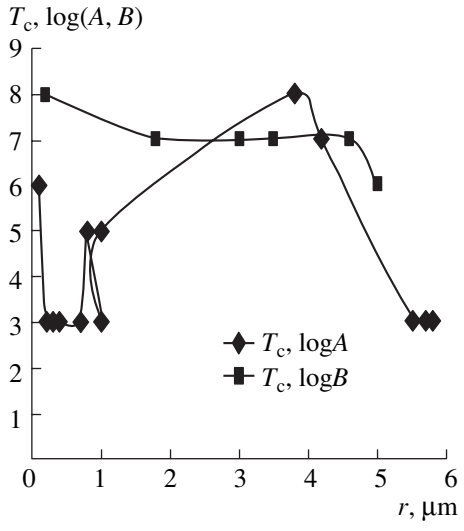

Fig. 7. The dependence of the titer of adsorbed *Acinetobacter* sp. (log$A$) and *B. mucilaginosus* (log$B$) cells on the predominant radius of adsorbent pores.

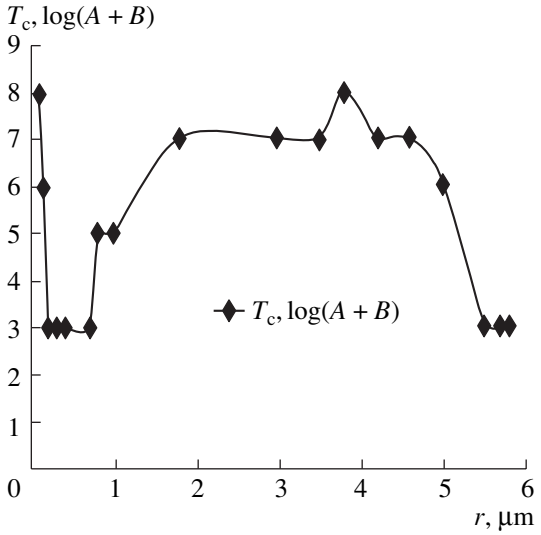

Fig. 8. The dependence of the total number of adsorbed *Acinetobacter* sp. and *B. mucilaginosus* cells on the predominant radius of adsorbent pores.

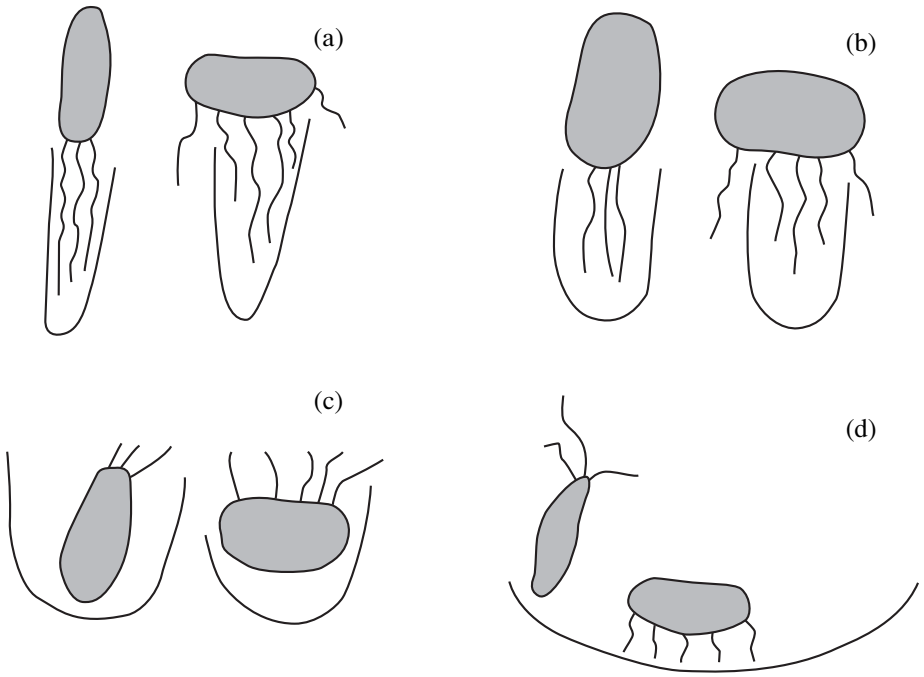

Fig. 9. Schematic representation of the attachment of microbial cells to the pore surface: (a) the flagella of cells are firmly attached to the pore surface; (b) the flagella of cells are loosely attached to the surface of large pores; (c) cells are firmly attached to adsorbent pores due to their comparable sizes; (d) cells are loosely attached to the surface of a large pore because of its small curvature.

1–1.5 μm and multiply by fission. The optimal pore radius for the adsorption of these cells is 2.0–4.5 μm, i.e., 2–5 times larger than the cell size. The optimal pore radius for the adsorption of *B. mucilaginosus* cells, which are 1.2–1.4 × 4–7 μm in size, is 3–4 μm, i.e., 2–3 times larger than the cell size.

Now let us explain the good adsorption of *Acinetobacter* sp. and *B. mucilaginosus* cells on adsorbents whose pores are smaller than the cells (Fig. 8). If the adsorbent pores cannot accommodate entire microbial cells, their adsorption is possible by means of flagella (*Acinetobacter* sp. and *B. mucilaginosus* cells have flagella 3–15 μm in length and 10–20 nm in thickness). In the case of adsorbents with narrow pores ($r <$ 0.3 μm), microbial cells are adsorbed with the aid of their flagella. With increasing pore size ($0.3 < r < 1$ μm), the cell titer T_c decreases. This is because the cells still cannot penetrate into adsorbent pores, whereas the large difference between the sizes of pores and flagella prevents the efficient attachment of the latter. With a further increase in the pore radius ($2 < r < 5$ μm), the size of adsorbent pores becomes comparable to that of microbial cells. The pores become able to accommodate whole microbial cells, and their adsorption considerably increases. Still larger adsorbent pores ($r > 5$ μm) do not favor cell adsorption since the small curvature of the pore interior diminishes the interaction of cells with the internal pore surface. Figure 9 gives a schematic illustration of the immobilization of two kinds of microbial cells on adsorbents with different pore sizes. The adsorption of microbial cells is good when the size of adsorbent pores is comparable to that of the cell flagella (Fig. 9a) or the cells themselves (Fig. 9c). When the pore size considerably differs from the sizes of the cells and flagella, the cell immobilization on adsorbents is inefficient (Figs. 9b, 9d).

Thus, the optimal values of the relevant parameters for the immobilization of microbial cells on porous adsorbents are as follows: The adsorbent hydrophilicity estimated with water vapor, *Ws* (H_2O), is between 0.07 and 0.24 g/g. The optimal radius of adsorbent pores for the adsorption of *Acinetobacter* sp. and *B. mucilaginosus* cells is 2–4.5 and 3–4 μm, respectively. The minimal specific area and the volume of macropores must be 0.001 m^2/g and 0.001 cm^3/g, respectively. The relatively high adsorption of microbial cells on adsorbents with narrow pores (<0.3 μm in radius) suggests that the cell flagella are involved in cell adsorption.

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