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Biodegradation of Polymeric Materials. Generalized Kinetic Data

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Information about generalized kinetic data which can describe biodegradation of polymeric materials (kinetics of biomass growth and methods of investigation of its formal mechanism of biodegradation, microorganism adhesion) is presented and discussed.

Keywords Adhesion; biodegradation; biomass growth; kinetics; methods of investigations; polymeric materials

Introduction

In the seventies-eighties academician N. M. Emanuel has drawn attention of the scientific community to the necessity of assessing the characteristics of time-dependent macroscopic processes (should it be the growth of tumor cells or aging of polymeric materials under action of the ambient medium) using the so-called generalized kinetic curves requiring a great body of experimental data. In particular, this approach was for the first time developed by N. M. Emanuel in his fundamental monograph [1] for quantitative description of the tumor-cell growth.

Biodegradation of polymers occurs when living organisms are in contact with these materials, it changes their operational properties [2,3]. Generally, the following processes can take place in biologically damaged polymers:

- adsorption on the material surface of microorganisms or species from tissues of living organisms;
- material degradation due to either specific action (living organisms use polymeric materials as the culture medium) or under action of the metabolism products.

In the first process, the chemical structure of the polymer is not changed as a rule, the material serves as a support on which microorganism colonies adhere

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and grow (bio-overgrowing) or collagenic capsules are formed with the implanted material. Microorganism adhesion is the initial stage of the material overgrowth which predetermines the further behavior of bio-overgrowth and bio-damage of polymeric materials. Assessment of the biomass on the polymer surface at the bio-overgrowth stage is of great interest, because the biomass amount influences the "surface" operational properties, such as optical, adhesion, and so on.

The second process leads directly to polymer aging under action of chemically active species. In this case, the "bulk" operational properties, such as mechanical, dielectric, and so on, will change.

In this paper we employed the method of generalized kinetic curves to assess the growth of microscopic fungi on the surface of polymeric material, to study the subsequent process of material biodegradation directly by the biological medium of organism or by the products of living activity of growing microorganisms (metabolites), and to estimate the kinetic parameters of microorganism adhesion on the polymer surface.

Kinetics of Biomass Growth. The Methods and Results

The growth and development of microscopic fangi directly on solid polymeric surfaces are conventionally assessed from the data on the growth of the diameter of certain colonies or of the colonies of some microscopic fangi using the five-grade scale and standard methods. This is because measurements of biomass in amounts of a few μg/cm^2 at the initial growth stage presents serious experimental difficulties.

To determine the kinetic parameters of biomass buildup, we used a sensitive radioisotopic method [4,5]; polymeric plates contaminated by a suspension of microscopic fangi in water or Chapek-Dox culture medium with a concentration of 10^6 cell/ml were held in the atmosphere of tritium water vapor. Tritium is accumulated in the biomass proportionally to the growing biomass containing on average 85 wt.% of water [6,7]. The biomass value was measured as a difference between the weights of the contaminated and reference polymer samples. The radiation intensity was measured on a Mark-388 liquid- phase scintillation counter.

Spread of microorganisms was assessed from changes in the amount of dry biomass per unit surface of a sample.

Figure 1 shows the kinetics of biomass buildup on various polymeric surfaces. The biomass variations on all the polymers studied are similar and are satisfactorily described by an empirical equation of the type of

$$\frac{\mathbf{m}}{\mathbf{m}_\infty} = 1 - \exp(-\mathbf{kt}), \tag{1}$$

where **m** is the instantaneous biomass, $\mathbf{m}_\infty$ is the equilibrium biomass, and **k** is the effective rate constant for biomass growth.

Table 1 lists the values of $\mathbf{m}_\infty$, the initial rate of biomass growth $\mathbf{V}_{\mathrm{init}}$, and $\mathbf{k}_{\mathrm{eff}}$ determined from the logarithmic plot Eq. (1) for two sorts of spore suspensions – in water and in the Chapek-Dox culture medium. The values of $\mathbf{m}_\infty$ and $\mathbf{V}_{\mathrm{init}}$ for the suspensions in the culture medium is twice as higher as that in water, while the $\mathbf{k}_{\mathrm{eff}}$ for these two media are nearly equal. The higher (as compared to other polymers) bio-overgrowth on cellulosic polymers is noteworthy. This is associated with

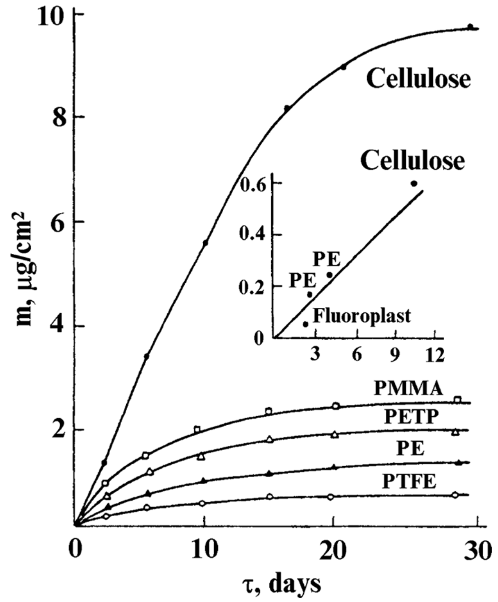

Figure 1. The kinetics of growth of microscopic fungi (the standard GOST set) on the surface of different polymers at $\varphi = 98\%$ and $T = 30°C$ (PMMA is polymethylmethacrylate, PETP is polyethyleneterephthalate, PE is polyethylene, PTFE is polytetrafluoroethylene). The inset shows the rate constant for the biomass growth as a function of the amount of water sorbed by the polymer.

their hydrophily. Indeed, the $\mathbf{k}_{\text{eff}}$ and $\mathbf{m}_\infty$ values are the greater, the more soluble the polymer in water (see inset in Fig. 1), which enables the biomass growth to be predicted for polymers with known water solubilities in the polymer. The generalized kinetic curve plotted in the $\frac{\mathbf{m}}{\mathbf{m}_\infty} - \mathbf{kt}$ coordinates is presented in Figure 2. It follows from this figure that all the biomass values fit a single curve whatever the nature of polymers, culture media, and microorganism types.

Similar dependences are also obtained for inhibited biomass growth in the presence of biostabilizers referred to as biocides [8].

The generalized kinetic plot for the buildup of *Aspergillus niger* microscopic fungus in the presence of various biocides is displayed in Figure 3, where the relative biomass amount is described by the following empirical equation

$$\frac{\mathbf{m}}{\mathbf{m}_\infty} = (1 + \mathbf{e}^{\theta})^{-1}, \tag{2}$$

where $\theta = \ln \mathbf{a} + \ln \mathbf{b}$; $\mathbf{a}$ and $\mathbf{b}$ are constants specifying biocide affinity.

Biodegradation

As mentioned above, biomass buildup or long-time contact of a material with a biological medium is attended by degradation processes.

Bioaging of polymers is most frequently appraised from variations of the mechanical properties of the samples, e.g., breaking stress $\boldsymbol{\sigma}$. For the majority of polymers

Table 1. The initial rate of the biomass buildup $\mathbf{V}_{\text{init}}$, and the limiting biomass value $\mathbf{m}_{\infty}$ on the surface of various polymeric materials

	Treating by a mixture of spores in the Chapek-Dox medium			Treating by a mixture of spores in water		
Material studied	$\mathbf{m}_{\infty}$, μg/cm^2	$\mathbf{V}_{\text{init}}$, μg/cm$^2 \cdot$ day	$\mathbf{k}_{\text{eff}} \cdot 10^6$, s^{-1}	$\mathbf{m}_{\infty}$, μg/cm^2	$\mathbf{V}_{\text{init}}$, μg/cm$^2 \cdot$ day	$\mathbf{k}_{\text{eff}} \cdot 10^5$, s^{-1}
Cellophane	10.5 ± 1	0.60 ± 0.05	1.0	5.80 ± 0.60	0.40 ± 0.080	1.5
Polyethylene terephthalate	2.4 ± 0.15	0.16 ± 0.02	1.2	0.27 ± 0.02	0.027 ± 0.003	0.9
Polyethylene	1.5 ± 0.20	0.27 ± 0.02	0.9	0.27 ± 0.02	0.01 ± 0.001	1.3
Polytetrafluoroethylene	1.1 ± 0.1	0.04 ± 0.01	0.7	0.17 ± 0.02	0.01 ± 0.001	0.8
Polymethylmethacrylate	—	—	—	0.91 ± 0.50	—	—
Polyimide	—	—	—	0.05 ± 0.01	—	—

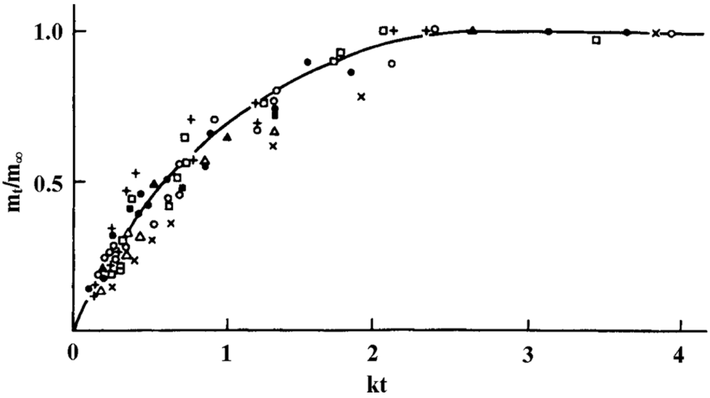

Figure 2. The generalized kinetic curve of the growth of *Aspergillus niger* fungus on the surface of various polymers at a humidity of 90% and T = 30°C. The fungus layer is applied from the aqueous suspension: ● – PE, o – PMMA, × – PETP, □ – cellulose, ■ – PTFE; from the Chapek-Don medium: + – PE, Δ – PETP, ▲ – PMMA.

the correlation between σ and the reciprocal average-number polymerization degree ($\overline{\mathbf{P}}_\mathbf{n}$) is linear:

$$\sigma = \mathbf{A} - \frac{\mathbf{P}}{\overline{\mathbf{P}}_0}. \tag{3}$$

For polymeric implanted materials degrading according to the law of random processes we can derive a simple formula relating $\boldsymbol{\sigma}$ to the implantation time:

$$\frac{\sigma}{\sigma_0} = 1 - \frac{\mathbf{k}}{\sigma_0}\mathbf{t}. \tag{4}$$

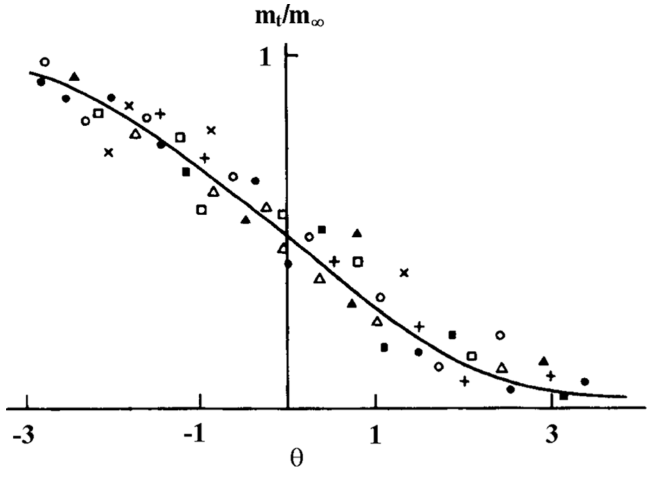

Figure 3. Generalized kinetics of inhibited biomass growth in the presence of biocides: o – Ionol, ● – Phlamal, □ – Merthiolate, Δ – $CuSO_2$, ■ – nitsedin, + – oxyphenyl, ▲ – alkylbenzyl-dimethylammonium chloride (ABDM), × – *N-para*-tolylmaleimide.

Denoting the time of complete strength loss by

$$\tau = \frac{\sigma_0}{\mathbf{k}}, \tag{5}$$

yields

$$\frac{\sigma}{\sigma_0} = 1 - \mathbf{t} \cdot \tau. \tag{6}$$

Figure 4 displays the generalized plot in the coordinates of Eq. (6) for bioaging of various medical polymers in the subcutaneous tissue of rabbits [9,10]. Thus, upon estimating the value of k from the initial portions of the kinetic curves, one can calculate by Eq. (5) the time of complete loss of the strength of the material implanted in a living organism.

The relative change in the breaking stress of elementary fibers of a canvas tissue upon incubation in it of a suspension of *Aspergillus niger* spores ($\mathbf{C}_0 = 10^6$ spore/ml) is shown in Figure 5. The value of $\boldsymbol{\sigma}$ drops linearly with time due to material degradation on the fiber surface:

$$\frac{\sigma}{\sigma_0} = 1 - \frac{\mathbf{k}_{\mathrm{eff(surf)}}}{\mathbf{l}_0 \rho}, \tag{7}$$

where $\boldsymbol{\rho}$ is the density, and $\mathbf{l}$ is the film thickness or fiber diameter.

Biodegradation of any particular polymer in the medium of a living organism is quantitatively specified by effective rate constants for cleavage of different bonds under the effect of hydrolizing factors. Previously in [10,11] we have shown that polyethylene terephthalate undergoes bulk degradation only in an acidic medium of the organism (e.g., when inflammation processes take place in it) with a rate constant of $1.3 \cdot 10^{-6}\,\mathrm{s}^{-1}$, whereas polycaproamide degrades via the mixed

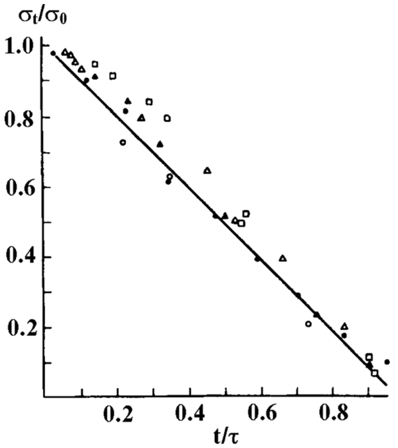

Figure 4. Generalized dependence of the relative change in the broking stress of various polymers implanted into the subcutaneous tissue of rabbits: ● – PE, Δ – polyglycolide, ▲ – polyglactine, □ – polyamide, o – polypropylene.

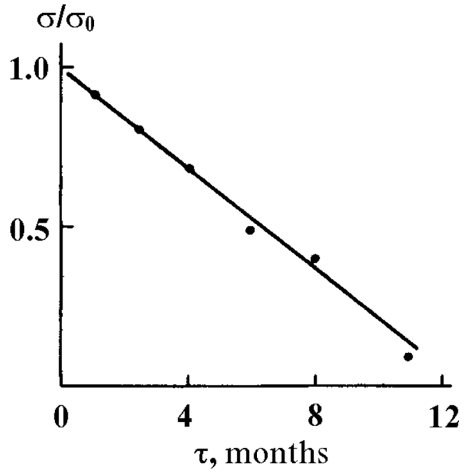

Figure 5. Relative values of the breaking stress for fibers of a woven cellulose-based cloth as a function of time during which microscopic *Aspergillus niger* fungi at a concentration of 10^6 cell/ml act on the sample.

mechanism (both on the surface and in the bulk) with rate constants of $0.15 \cdot 10^{-8}\,\mathrm{s}^{-1}$ and $10^{-1}\,\mathrm{s}^{-1}$, respectively.

In the final run, each particular polymer must be characterized by a set of effective degradation rate constants, as listed in Table 2.

Kinetics of Microorganism Adhesion

Biodegradation, as a result of microorganism interaction with the polymer surface, starts with the adhesion process. The adhered cells act as aggressive bioagents by virtue of exoenzymes or other low-molecular species evolved by the organism. Therefore the quantitative adhesion parameters govern the rates of bio-overgrowing (biomass build up) and biodegradation [12].

The adhesion strength ($\mathbf{F}_{\mathrm{adh}}$) as measured by the centrifugal break-off technique is a parameter amenable to quantitative analysis. To estimate the value of $\mathbf{F}_{\mathrm{adh}}$, suspension of microorganism cells (10^6 spore/ml) was applied to the polymer film surface and incubated for some time period under various external conditions

Table 2. Effective rate constants for degradation of biomedical polymers in various media of living organisms*

Polymer	$\mathbf{k}_{\mathrm{acid}}$, s^{-1}	$\mathbf{k}_{\mathrm{sal}}$, s^{-1}	$\mathbf{k}_{\mathrm{H_2O}}$, s^{-1}	$\mathbf{k}_{\mathrm{enz}}$, s^{-1}
Polycarpoamide	—	$0.15 \cdot 10^{-8}$	10^{-10}	—
Polyglicolide	—	$1.30 \cdot 10^{-4}$	$0.8 \cdot 10^{-3}$	$5.00 \cdot 10^{-4}$
Polyglactine	—	$5.00 \cdot 10^{-4}$	$0.9 \cdot 10^{-3}$	$0.13 \cdot 10^{-4}$
PETP	$1.3 \cdot 10^{-6}$	—	—	—
Polyethylene	—	—	—	$1.2 \cdot 10^{-9}$ [11]
Cellulose	—	—	—	$0.5 \cdot 10^{-6}$

*The data obtained by the authors.

(temperature 7°C and humidity φ, %). Then the films were fastened on metal plates and centrifuged in the field of forces directed normally to the surface. The number of cells (γ) torn from the surface in a given field of forces and collected in the centrifuge cup filled with distilled water were counted under microscope. Adhesion was quantified by the force of spore break-off of the polymer surface:

$$\mathbf{F} = \left(\frac{1}{675}\right)\pi^3 \mathbf{r}^3 \omega^3 \mathbf{R}(\rho_{\mathrm{cell}} - \rho_{\mathrm{liq}}), \tag{8}$$

where $\mathbf{r}$ is the spore radius, $\boldsymbol{\omega}$ is the angular rotation velocity, $\mathbf{R}$ is the distance from the rotor axis, $\boldsymbol{\rho}_{\mathrm{cell}}$ is the spore (cell) density, and $\boldsymbol{\rho}_{\mathrm{liq}}$ is the density of a liquid in which the spores are torn off.

Typical dependences of γ_F on the time of microorganism spore holding on the surface are S-shaped adhesion curves specified by two parameters: the equilibrium adhesion number under given conditions, γ_∞, and the rate constant of formation of the adhesion forces, $\mathbf{k}_{\mathrm{eff}}$, which is determined from the expression describing the experimental curves:

$$\ln\frac{\gamma}{\gamma_\infty} = -\mathbf{k}_{\mathrm{ads}}\mathbf{t}. \tag{9}$$

The effect of the material type on adhesion of *Aspergillus niger* spores is shown in Figure 6 which displays the time histories of adhesion under certain thermal and humidity conditions for various polymers. The adhesion curves level off in 24 h. The calculated rate constants for formation of the adhesion forces γ_∞, and the adhesion forces themselves are listed in Table 3.

Polymers can be arranged in the order of increasing spore adhesion from hydrophobic polyethylene to polyethylene-terephthalate.

From the experimental data on the numbers of *Aspergillus niger* spore adhesion at a constant humidity at various temperatures, we calculated the parameters for two polymers with extreme values of water solubility: polyethylene and cellophane (Table 4).

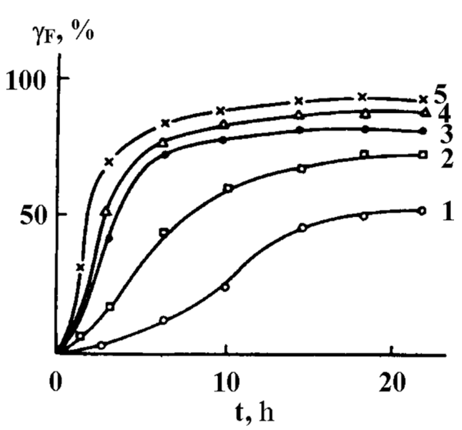

Figure 6. Kinetics of adhesion of *Aspergillus niger* cells at 22°C and $\varphi = 98\%$ to the surface of various polymers: (1) PE; (2) epoxy-polymer; (3) PMMA; (4) acetylcellulose; and (5) cellophane.

Table 3. Parameters of *Aspergillus niger* spore adhesion to the surface of various polymeric materials

Material	**k**, h^{-1}	$\mathbf{F}^{50}$, dyn/cell	γ_∞, %
Polyethylene	0.06	$3.3 \cdot 10^{-4}$	55
Epoxy resin	0.08	$6.6 \cdot 10^{-4}$	70
Polymethylmethacrylate	0.36	$1.0 \cdot 10^{-3}$	80
Cellophane	0.36	$1.6 \cdot 10^{-3}$	85
Acetylcellulose	0.41	$2.5 \cdot 10^{-3}$	90
Polyethyleneterephthalate	0.41	$2.5 \cdot 10^{-3}$	90

Table 4. Adhesion parameters of the interaction for polyethylene and cellophane at various temperatures and a relative humidity $\varphi = 30\%$

	Polyethylene			Cellophane		
T,°C	γ_∞, %	**k**, h^{-1}	$\mathbf{F}^{50}$, dyn/cell	γ_∞, %	**k**, h^{-1}	$\mathbf{F}^{50}$, dyn/cell
10	85	0.06	$5.2 \cdot 10^{-4}$	90	0.36	$2.6 \cdot 10^{-3}$
22	55	0.06	$3.3 \cdot 10^{-4}$	85	0.36	$1.6 \cdot 10^{-3}$
38	50	0.06	$1.3 \cdot 10^{-4}$	—	—	—

Table 5. Adhesion parameters for *Aspergillus niger* spores on polyethylene at various air humidity and a constant temperature T = 10°C

φ, %	**k**, h^{-1}	γ_∞, %	$\mathbf{F}^{50}$, dyn/cell
0	0.08	70	$3.0 \cdot 10^{-3}$
30	0.66	85	$5.2 \cdot 10^{-4}$
100	0.56	100	$1.9 \cdot 10^{-3}$

The temperature dependence of adhesion is not profound for each polymer, which is supported by the invariable rate constants for formation of adhesion forces (Table 4). In addition, the adhesion force increases with decreasing temperature.

Table 6. Adhesion parameters for microscopic fungi on the polyethylene surface at φ = 98% and **T** = 22°C

Fungus type	γ_∞, %	**k**, h^{-1}	**r**, μm	$\mathbf{F}^{50}$, dyn/cell
Aspergillus niger	80	0.23	5.0 ± 0.5	$0.26 \cdot 10^{-1}$
Aspergillus terreus	94	1.96	1.0 ± 0.05	$0.31 \cdot 10^{-3}$
Paec. varioti	98	0.30	5.5 ± 0.7	$0.76 \cdot 10^{-1}$
Penicillium Chrisogenum	94	0.40	1.5 ± 0.06	$0.45 \cdot 10^{-3}$
Penicillium cyclopium	55	0.50	2.6 ± 0.4	$0.10 \cdot 10^{-1}$

Note. **r** is the cell (spore) radius of microscopic fungi.

Table 7. Parameters of polymer biodegradation

	Adhesion parameters			Growth				
Material	$\mathbf{k}_{\mathrm{eff}}^{\mathrm{adh}}$, s^{-1}	F^{50}, dyn/cell	γ_∞, %	$\mathbf{m}_\infty$, $\mu g/cm^2$	$\mathbf{k}_{\mathrm{acid}}^{\mathrm{eff}}$, s^{-1}	$\mathbf{k}_{\mathrm{sal}}^{\mathrm{eff}}$, s^{-1}	$\mathbf{k}_{\mathbf{H_2O}}$, s^{-1}	$\mathbf{k}_{\mathrm{enz}}$, s^{-1}
Polyethylene (LPPE)	$1.6 \cdot 10^{-5}$	$3.3 \cdot 10^{-4}$	55 ± 5	1.5 ± 0.2	—	—	—	$1.2 \cdot 10^{-9}$
Epoxy polymer	$2.2 \cdot 10^{-5}$	$6.6 \cdot 10^{-4}$	70 ± 5	4.5 ± 0.7	—	—	—	$1.7 \cdot 10^{-7}$
Polymethyl-methacrylate	$2.1 \cdot 10^{-5}$	$1.1 \cdot 10^{-3}$	80 ± 5	3.8 ± 0.7	Was not studied	Was not studied	Was not studied	Was not studied
Polyethylene terephthalate	$1.1 \cdot 10^{-4}$	$2.5 \cdot 10^{-3}$	90 ± 5	2.4 ± 0.5	$-1.3 \cdot 10^{-6}$	—	—	—
Cellulose	$1.0 \cdot 10^{-4}$	$1.6 \cdot 10^{-3}$	85 ± 5	10.5 ± 1.0	—	—	—	$0.5 \cdot 10^{-6}$
Polyimide	$1.7 \cdot 10^{-4}$	$1.9 \cdot 10^{-3}$	82 ± 5	3.9 ± 0.5	—	$0.2 \cdot 10^{-8}$	10^{-10}	—

Note. $\mathbf{k}_{\mathrm{eff}}^{\mathrm{adh}}$ is the rate constant for adhesion of microscopic *Aspergillus niger* fungus; **F** is the force of a single cell adhesion to the polymer surface; γ_∞ is the limiting adhesion number; $\mathbf{m}_\infty$ is the biomass per unit surface area; $\mathbf{k}_{\mathrm{acid}}^{\mathrm{eff}}$ is the mass rate constant for degradation in an acidic medium; $\mathbf{k}_{\mathrm{sal}}^{\mathrm{eff}}$ is the mass rate constant for degradation under effect of phosphate ions; $\mathbf{k}_{\mathrm{enz}}^{\mathrm{eff}}$ is the rate constant of the degradation induced by enzymes [13].

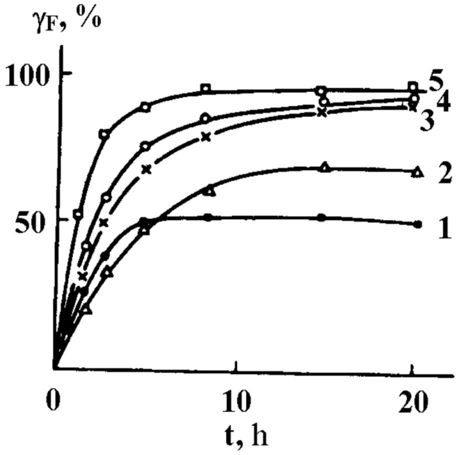

Figure 7. Kinetics of adhesion of various microscopic fungi to the polyethylene surface: (1) *Aspergillus niger*; (2) *Pen. cyclopuim*; (3) *Paec. varioti*; (4) *Pen. chrysogenum*; and (5) *Aspergillus terreus*.

From the kinetic curves for adhesion of *Aspergillus niger* spores to the polyethylene surface at a constant temperature and various humidity of the medium we calculated the rate constants; it follows from the analysis of these calculations that the **k** values change appreciable, whereas γ_∞ values differ insignificantly (Tables 5 and 6).

The adhesion parameters were calculated (Table 7) from the kinetic curves measured for adhesion of various microscopic fungi to the polyethylene surface shown in Figure 7.

The final goal of investigations into the quantitative parameters of adhesion leading to bio-overgrowth is evaluation of the kinetic parameters for polymer biodamage. The experimental data are included in the summarizing table, from which one can infer that the biomass adhesion and growth are related by a linear dependence; as to the degradation process, it calls for additional studies in model media to determine the degradation type. The adhesion number γ_∞ for various fungi ranges between 98 and 55.

The experimental values of the kinetic parameters characterizing interactions of microorganism cells (spores) with a polymer surface make it possible to forecast biological stability of the material. Generally, biological stability (**B**) depends on the adhesion force, biomass amount, and effective rate constants for cleavage of the accessible bonds:

$$\mathbf{B} \propto \frac{1}{\mathbf{F}_{\text{ads}}} \frac{1}{\Delta \mathbf{m}_\infty} \frac{1}{\mathbf{k}_{\text{eff}}}. \tag{10}$$

Thus, it is shown that investigations into the kinetics of such macroscopic processes as adhesion, bio-overgrowth, and bio-degradation (Table 7) allows the mechanism of complex processes responsible for biostability and biodegradation of polymeric materials to be modelled.

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