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BIODEGRADATION OF POLYMERIC MATERIAL AND ADHESIVE PROPERTIES OF CELLS OF MICROORGANISMS

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Abstract. The chemical composition of cell membranes of microscopic fungi destroying polymeric materials is analyzed. The concentrations of the major chemical components – phospholipids, glycans, and amines – in nine types of fungi are estimated. The measured ratios of these components provide an estimate of the hydrophobic properties of conidia, controlling microorganism adhesion to polymer surface and subsequent degradation of polymeric materials.

Keywords: biodegradation, polymer, materials, adhesive, properties, cells, microorganisms.

1. Introduction

Biological stability of polymeric materials hinges on a variety of factors, in particular, on composition and properties of the contacting surfaces of polymers and microorganisms, controlling the early stage of a complex process – biodegradation. The strength and origin of adhesive bonds depend, in turn, on the chemical nature of substances secreted by cells of microorganisms, reducing biodegradation to conventional chemical decomposition. Cell membranes of microscopic fungi control most vital aspects of cell activity, from adhesion to cell fission and growth. This should be taken into account when predicting biological stability of polymeric materials which primarily depends on the ability of conidia – microstructural units of microscopic fungi – to adhere to surfaces.

The modern concept is that conidial membranes consist of several layers, the number and thickness of which depend on the conditions of their growth. A cell membrane contains polysaccharides (mostly, chitin and glycans), lipids, proteins and pigments [1, 3]. The basic method of exploring the composition of a cell membrane is chemical analysis which is labour-consuming and, in certain cases, gives no way of identifying chemical constituents of the membrane completely, because membranes are destroyed in the course of analysis.

In recent years, it has become usual to analyze the chemical composition of membranes by a non-destructive method, namely, by multiple attenuated total reflectance spectroscopy in the infrared spectral range (IR MATR), which was first successfully applied in [4-7] to qualitatively identify the chemical species contained in membranes of yeast cells.

This study was accomplished with an intent to determine quantitative composition of the surface layer of conidia by IR MATRS and correlate the chemical composition with hydrophobic properties of their cells.

2. Experimental

2.1. Materials

The objects of investigation were conidia of the following microscopic fungi: *Aspergillus niger*, *Aspergillus terreus*, *Penicillium chrysogenium*, *Penicillium funiculosum*, *Penicillium cyclopilun*, *Paecilomyces varioti*, *Thrichoderma viride*, *Chaetomicin globusum* and *Aspergillius flavus*. This set of microorganisms is incorporated in all-union state standard analysis of polymers for their biological stability.

2.2. Methods

The fungi were grown on an agarized Capek-Dox culture medium in test tubes at 303 K for 14 days. Conidia were washed off fungal populations with sterile distilled water onto "Synpor" membrane filters (the conidium layer was ~ 0.01 mm thick), following which the collected spores were rinsed with distilled water, dried in a $CaCl_2$ desiccator and analyzed by MATR spectroscopy. The filter with a conidium layer on it was settled on the MATR element and pressed to it (the strength with which the filter was pressed to the element was varied until it ceased affecting the intensity of IR bands). Spectra were taken on a

"Qualimatic" Fourier-transform IR spectrometer ("Digilab") with an MATR element made from KRS-5 crystal and germanium in the shape of a prism.

IR radiation penetrated through a depth estimated as

$$l = \frac{\Gamma_i/n}{2p\left[\sin^2 q - (n_s - n_0)^2\right]^{1/2}} \tag{1}$$

where Γ_i is the radiation wavelength; n_s is the refractive index of the crystal; n_0 is the refractive index of the polymer sample and q is the effective angle of incidence equal to 45°.

To calculate the depth of radiation penetration, one has to know the refractive index of conidial membranes. Refractive indexes of the chemical species contained in a membrane differ only slightly, and therefore, one was taken equal to ~ 1.5. In the spectral range from 2000 to 1500 cm^{-1} with the major absorption bands of the membrane constituents in it, radiation penetrates into a KRS-5 crystal through a distance ranging from 0.5 to 0.8 μm, and starting from 1500 cm^{-1}, the penetration depth increases drastically and attains 1.8 μm at 800 cm^{-1}; for germanium this dependence is much smoother: over the range from 2000 to 1500 cm^{-1}, the penetration depth increases from 0.1 to 0.2 μm, and at 800 cm^{-1}, it measures ~ 0.3 μm.

IR spectra of certain chemical components of a conidial membrane, namely, of melanin, chitin (extracted directly from *Aspergilius niger*), and glycans (Serva-made), were taken from their pellets (1-5 mg of a membrane component per 300 mg KBr). The spectra of phospholipids (of the lipid fraction extracted from *Aspergillus niger*) were taken in chloroform solutions in a 0.03-cm thick NaCI cell.

Hydrophobic properties of the conidium surface were judged from its wettability measured in terms of contact angles with an MG-1 horizontal microscope. To this end, we mounted a filter with a dense layer of conidia on it on the microscope table, dripped a drop of water of size 1-2 mm from a syringe on the surface of the layer, and measured the contact angle. The surface roughness was taken into account as follows:

$$\cos q = r \cos q_0 \tag{2}$$

where q is the observed macroscopic angle; q_0 is the true macroscopic angle; r is the surface roughness expressed as a ratio of the true area of the rough surface to its geometric area.

The dependence of the contact angle q on the surface roughness was reproduced from the data of [9]. The contact angle either increases or decreases with increasing surface roughness, depending on whether q exceeds 85° on a smooth surface, or is below 60°. In view of these tendencies, the contact angle would be expected to increase on the rigid spherical spores. However, contrary to the expectations, repeated measurements have not revealed any asymmetry of the drop edges, which would be observed, if protruding hemispheres affected the surface roughness. Therefore, we considered it justified not to introduce a correction for the roughness of the surface layer of the filtered conidia.

3. Results and Discussion

Representative MATR spectra of conidia of four fungal species are illustrated in Fig. 1. As revealed by the analysis of the measured spectra, the chemical constituents of a conidial membrane can be identified by the absorption bands recorded at 1639–1641, 1550–1560, 1275, 1033–1100 and 831–835 cm^{-1}. These bands were chosen, because:

• they overlapped very little, if at all, *i.e.* their positions and intensities were easy to determine;

• their intensities changed dramatically from one fungus type to another, which made them particularly handy for analyzing the quantitative composition of conidial membranes.
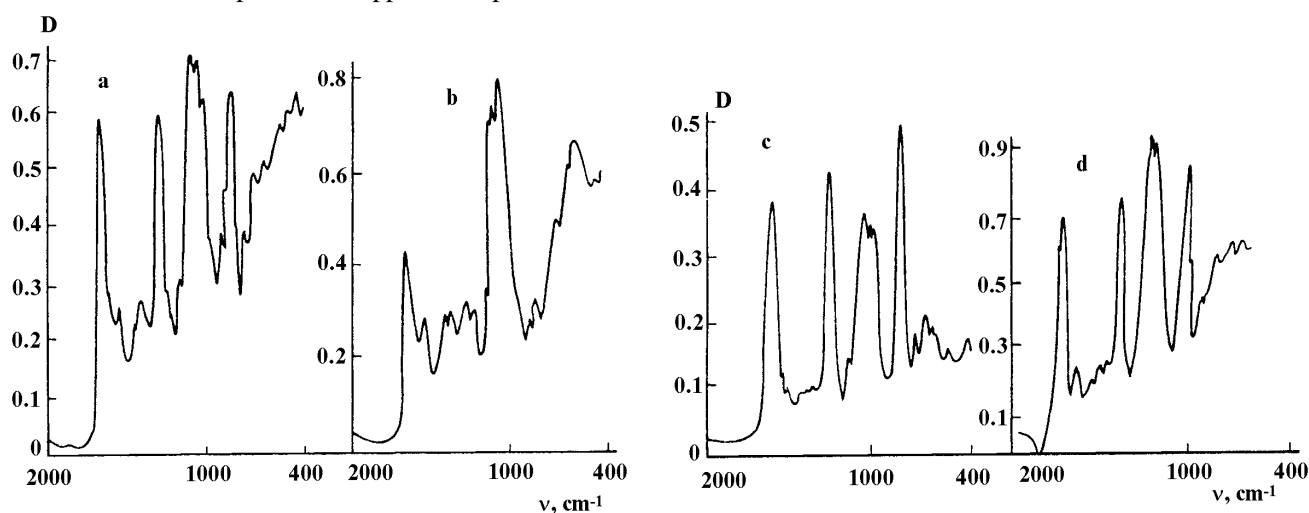

Fig. 1. IR spectra of cell membranes of the following microscopic fungi: *Thrinchoderma vitide*(a), *Paecilomyces vatioti*(b), *Penicillium funiculosum*(c) and *Aspergillus terreus*(d)

Absorption at 1639–1641 cm^{-1}. In this spectral range, strongly absorb proteins and glycans [6]. The absorption band of proteins, amide I, involves stretching vibrations of C=O (~ 80%), stretching vibrations of C–N (~ 10 %), and bending vibrations of N–H of amide groups (~ 10 %). From the position of the amide I band one can infer the conformation of a protein molecule; absorption at 1656, 1650, and 1637 cm^{-1} testifies to a disordered structure (to the *a*-helix and *b*-structure of protein molecules). Absorption in the neighborhood of 1639 cm^{-1} is also inherent in *b*-glycans separated out from cell membranes of the fungi [10, 11].

Absorption at 1275 cm^{-1} This absorption band is due to stretching vibrations of P=0 fragments of phospholipids. Two types of phosphate groups are distinguished, the groups bound to proteins with hydrogen bridges and the free ones: the former absorb at 1350–1220 cm^{-1}, and the latter at 1300–1250 cm^{-1}. Attention is arrested by the fact that the 1275 cm^{-1} band appeared in the spectra of all the fungi, which indicates the presence of free phosphate groups in them.

Absorption at 1550–1540 cm^{-1} is due to bending vibrations of NH groups (amide II) occurring in both proteins and chitin.

Absorption at 831 cm^{-1} associated with out-of-plane bending vibrations of C–H groups indicates the presence of *a*-glycans [12]. It has been shown that various *a*-glycans (mannans, nigerans) isolated from cell membranes of the fungi exhibit absorption peaks in the range from 850 to 830 cm^{-1}.

Absorption at 1070–1050 cm^{-1} [4] associated with stretching vibrations of CO groups is inherent in glycans and proteins [4, 5, 10].

The baseline required to measure band intensities was drawn through the lowest points of peak bases. Extinction coefficients of the membrane constituents at the aforesaid frequencies were calculated from absorption spectra taken at different concentrations of individual substances (chitin, proteins, phospholipids and glycans).

Optical densities of nine species of the microscopic fungi are listed in Table 1.

Concentrations of the chemical components of conidial membranes were estimated under the following assumptions:

• the Lambert-Bouguer-Bragg law in the form

$$C = \frac{D}{le}$$

was observed for all the membrane components (here, D is the optical density, e is the extinction coefficient, and l is the depth of radiation penetration);

• extinction coefficients of individual substances (chitin, proteins, phospholipids and *a*-glycan) assume identical values in all the microscopic fungi;

• to avoid errors in reflectance spectra, we used ratios of optical densities of different absorption bands in all instances;

Table 1

Optical densities of the principal absorption bands of conidial membranes of the microscopic fungi

No.	Microscopic fungus	Optical density, arb. units				
		1639–1641 cm^{-1}	1550–1560 cm^{-1}	1275 cm^{-1}	1033–1100 cm^{-1}	831–835 cm^{-1}
1	*Penicillium funiculosum*	0.35 ± 0.02	0.01	0.36 ± 0.03	0.28 ± 0.02	0.39 ± 0.03
2	*Penicillium chrysogenium*	0.26 ± 0.02	0.05 ± 0.01	0.13 ± 0.02	0.53 ± 0.03	0.13 ± 0.02
3	*Thrichoderma viride*	0.50 ± 0.03	0.06 ± 0.01	0.38 ± 0.04	0.50 ± 0.03	0.41 ± 0.03
4	*Aspergillus niger*	0.30 ± 0.02	0.08 ± 0.01	0.20 ± 0.02	0.40 ± 0.03	0.14 ± 0.02
5	*Aspergillus terreus*	0.64 ± 0.03	0.07 ± 0.01	0.56 ± 0.06	0.68 ± 0.04	0.50 ± 0.05
6	*Chaetomicin globusum*	0.26 ± 0.02	0.05 ± 0.01	0.13 ± 0.02	0.63 ± 0.03	0.10 ± 0.02
7	*Aspergillius flavus*	0.35 ± 0.02	0.05 ± 0.01	0.16 ± 0.02	0.33 ± 0.02	0.12 ± 0.02
8	*Penicillium cyclopilun*	0.28 ± 0.02	0.05 ± 0.01	0.17 ± 0.03	0.58 ± 0.03	0.17 ± 0.02
9	*Paecilomyces varioti*	0.33 ± 0.02	0.08 ± 0.01	0.13 ± 0.01	0.55 ± 0.03	0.08 ± 0.02

• mass fractions of the major membrane components added up to unity $C_a + C_l + C_g = 1$ (where C_a stands for the species containing amide groups, *e.g.* proteins and chitin, C_l – for phospholipids, and C_g – for *a*-glycans).

The concentration of phospholipids, *a*-glycans, and other substances containing amide groups were calculated from the ratios of optical densities in the characteristic absorption bands of these species (at 1275 cm^{-1} for phospholipids, at 831–835 cm^{-1} for *a*-glycans, and at 1550–1560 cm^{-1} for amides):

$$\frac{D_g}{D_l} = AX, \qquad \frac{D_g}{D_l} = BY \qquad (3)$$

where $A = \left(\frac{l_g}{l_l}\right)\left(\frac{e_g}{e_l}\right)$, $B = \left(\frac{l_a}{l_l}\right)\left(\frac{e_a}{e_l}\right)$, $X = \frac{l_g}{l_l}$ and

$Y = \frac{C_a}{C_l}$.

From the balance equation we obtain

$$C_l = \frac{1}{X + Y + 1}, \; C_a = \frac{Y}{X + Y + 1}, \; C_g = \frac{X}{X + Y + 1} \qquad (4)$$

From the aforesaid absorption bands we derived the following estimates:

$$\frac{l_g}{l_a} = 1.80, \; \frac{e_g}{e_l} = 1.6 \pm 0.2, \; \frac{l_a}{l_l} = 0.20, \; \frac{e_a}{e_l} = 0.33 \pm 0.03$$

The calculated concentrations of phospholipids, *a*-glycans, and amide-containing species (chitin, proteins) are summarized in Table 2.

Absorption bands at 1639-1641 and 1033-1100 cm^{-1} are composite, *i.e.*, consist of absorption bands of several groups:

$$D = \sum_i (e_i c_i) l \qquad (5)$$

As revealed by the analysis of the experimental data, these bands involve absorption of amide groups and *a*-glycans.

The total concentrations of the major chemical components in cell membranes of the nine species of microscopic fungi calculated from our data vary over fairly wide ranges: *a*-glycans 0.05–0.25, phospholipids 0.26–0.72, and amides 0.01–0.69.

Ratios of membrane components in cells of the fungi, available from the literature, are also widely spread. According to [10], *e.g.*, the concentrations of glycans in the outer and inner membranes *of Cimitic conidia* related as 3 : 2, which suggests that glycans are mostly contained in the outer membrane. However, the authors of [12] argue that the outer membrane of certain fungal species contains 50 % proteins, 25 % phospholipids, and less than 15 % glycans. We have failed to find any data on the lipid content of fungal membranes in other papers on the subject, which are mostly concerned with the glycan-to-chitin ratio estimated at 6 : 1 [12]. The data gleaned by analysis of conidial membranes are widely spread, primarily because the test samples were variously prepared: by disintegration, dissolution, and biochemical reactions. From NMR study of five species of gramm-positive bacteria it has been concluded [13] that cell membranes of bacteria mostly consist of peptide glycans.

As intimated earlier in this paper, the wettability of fungal membranes in water is estimated in terms of contact angles. To correlate q to the membrane composition of each fungus, we constructed the equation relating q to the sum of total concentrations of the membrane components taken with the appropriate weight factors (*a, b, g*):

$$\log q_i = aC_a + bC_l + gC_g \qquad (6)$$

Table 2

Concentrations of phospholipids, *a*-glycans, and amide-containing components in conidial membranes of the microscopic fungi

No.	Microscopic fungus	Mass fraction			Contact angle, grad
		Amide	Phospolipid	*a*-Glycans	
1	*Penicillium funiculosum*	0.01	0.73	0.26	130
2	*Penicillium chrysogenium*	0.56	0.33	0.11	109
3	*Trichoderma viride*	0.28	0.54	0.18	90
4	*Aspergillus niger*	0.58	0.34	0.08	101
5	*Aspergillus terreus*	0.30	0.54	0.16	120
6	*Chaetomycin globusum*	0.57	0.35	0.08	115
7	*Aspergillus flavus*	0.52	0.39	0.10	130
8	*Penicillium cyclopium*	0.49	0.38	0.13	107
9	*Paecilomyces varioti*	0.69	0.26	0.05	90

By solving the correlation equations for nine types of fungi, we obtained the following weight factors allowing for the contributions of various chemical components to the surface properties of fungal membranes, responsible for their wettability in water, *i.e.* for hydrophobic properties of membranes:

$$a_a = 1.85 \pm 0.05, \quad b_l = 2.6 \pm 0.1, \quad \text{and} \quad g_g = 0.75 \pm 0.05.$$

Considering that b is 1.5–3.5 times greater than either of the other two coefficients, hydrophobic properties of the outer membrane mostly depend on phospholipids.

4. Conclusions

1. The chemical composition of cell membranes of microscopic fungi destroying polymeric materials is analyzed. The concentrations of the major chemical components – phospholipids, glycans, and amines – in nine types of fungi are estimated.

2. The measured ratios of these components provide an estimate of the hydrophobic properties of conidia, controlling microorganism adhesion to polymer surface and subsequent degradation of polymeric materials.

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БІОДЕГРАДАЦІЯ ПОЛІМЕРНИХ МАТЕРІАЛІВ І АДГЕЗІЙНІ ВЛАСТИВОСТІ КЛІТИН МІКРООРГАНІЗМІВ

Анотація. Проаналізовано хімічну структуру клітинних мембран мікроскопічних грибків, що руйнують полімерні матеріали. Для дев'ятьох типів грибків встановлені концентрації основних хімічних компонентів – фосфоліпідів, глісанів і амінів. Одержані дані дозволяють оцінити гідрофобні властивості конідії, що дає можливість контролювати її адгезію до полімерних поверхонь та подальшу деградацію полімерних матеріалів.

Ключові слова: біодеградація, полімер, матеріали, адгезія, властивості, клітини, мікроорганізми.