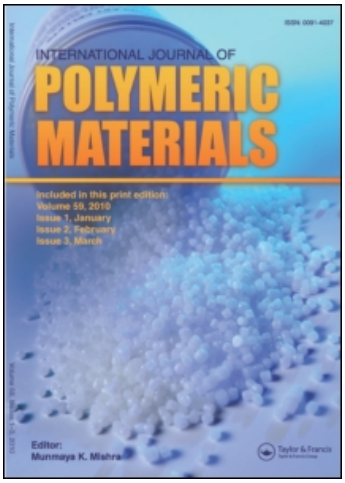

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Biodegradation of Polymeric Material and Adhesion Properties of Microorganism Cells

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This paper addresses the possibility of applying IR-spectroscopy with Fourier analysis to the study of the chemical composition of conidia films, defining surface hydrophobia. The possibility was obtained to predict conidium hydrophobia, based on the available data on component ratio. This is the defining factor at microorganism adhesion on solid surfaces and at further processes of biodegradation.

KEY WORDS Cell walls, fungi, composition, hydrophobia, IR spectroscopy.

INTRODUCTION

Biostability of polymeric materials depends on many factors, such as the composition and properties of interacting surfaces of polymer and microorganisms, and characterizing adhesion—the initial stage of complex biodegradation process. The reliability and character of adhesion bond is defined by the type of chemical substances, separated by microorganism cells, which finally conduct biodegradation process to chemical degradation.

Cell walls of microscopic fungi define lots of cell functions starting with adhesion to cell growth and division. All this is sufficiently important at the point of consideration of polymeric material biostability, depending, first of all, on the ability of microstructural unit of microscopic fungi—conidia—to adherence at material surface.

Modern ideas present the conidium wall as a combination of several layers, whose number and size depend on growth conditions.[1,2] The following compounds are contained in a cell wall: polysaccharides (mainly chitin and glycanes), lipids, proteins and pigments.[1,3]

The main method of investigation of conidium cell wall composition is chemical analysis. It is very labor intensive and does not lead to complete identification of chemical substances constituting the wall. This is because during analysis the wall is destroyed.

Recently, the internal refraction (NBRIG)† in IR-range was applied for identification of substances contained in the wall of yeast cells, and qualitative analyses

†ATR.

of compounds in the wall were performed for the first time by nondestructive method.[4-7]

The main scope of the present work was a quantitative determination of composition of substances, contained in conidium surface layer, and correlation between the chemical composition and cell hydrophobia by the NBRIG method in IR-range.

MATERIALS AND METHODS

Conidia of the following microscopic fungi were studied: *Aspergillus niger, Aspergillus terreus, Penicillium chrysogenium, Penicillium funiculosum, Penicillium cyclopium, Paecilomyces varioti, Thrichoderma viride, Chaetomicin globusum, Aspergillius flavus.* This list of microorganisms is provided by GOST‡ for estimation of polymers for biostability.

Fungi were grown in test-tubes with agarized Chapek-Docks culture medium at 30°C temperature during 14 days. Conidia were collected by washing them off from pillar using sterilized distilled water. Then, they were absorbed on membrane filters "Synpor" (conidium layer thickness ~0.01 mm), washed by distilled water and dryed in excicator on $CaCl_2$. The filter with conidium layer was placed on NBRIG element and pressed at increasing pressures until the pressure level influenced the intensity of IR-spectrum bands. The spectra were obtained using the Fourier IR-spectrometer "Qualimatic" of Digilab Company, with CRS-5 or germanium prisms as NBRIG element.

The depth "l" of IR-radiation penetration was determined by the formula:

$$l = \frac{\tau_i/n}{2\pi[\sin^2\theta - (n_s/n_0)^2]^{1/2}} \quad (1)$$

where τ_i = radiation wave length; n_s = crystal refractive index; n_0 = sample material refractive index; θ = efficient falling angle, equal 45°.

To determine the penetration depth one should know the refractive index of conidium wall. The refractive indexes of each group of substances contained in cell wall vary little. Therefore, we assumed that n_0 equals ~1.5. For KRS-5 crystal in the range of the main absorption bands of present substances, the penetration depth varies from 0.5 μm up to 0.8 μm (2000–1500 cm^{-1}). It increases sharply, starting from 1500 cm^{-1}, and reaches 1.8 μm at 800 cm^{-1}. For germanium crystal this dependence is smoother and "l" values vary from 0.1 to 0.2 μm at (2000–1500 cm^{-1}), and increase up to 0.3 μm at 800 cm^{-1}.

IR-spectra of the following substances, contained in conidium walls, were obtained using tablets (1–5 mg of substance per 300 mg of KBr): melanin, chitin, separated directly from *Aspergillus niger*, glycanes ("Serva" Company). Spectra of phospholipids (lipid fraction, separated from *Aspergillus niger*) were obtained using their chloroformic solution in a 0.03 cm thick NaCl-cuvette.

‡Russian standard.

Conidium surface hydrophobia was estimated by measuring the contact wetting angles with the help of a horizontal microscope MH-1. For this purpose, the filters with dense conidium layer were placed on the sample table. By placing a water drop of 1–2 mm size on the sample by a syringe the contact angle was measured. The degree of irregularity was calculated using[8]:

$$\cos \theta = r \cdot \cos \theta_0 \tag{2}$$

where θ = observed microscopic angle; θ_0 = real microscopic angle, r = surface irregularity, expressed as a ratio of real square of surface irregularity to the square of geometric surface. According to the data from Reference 9, the influence of irregularities of polymeric surface on contact angle θ was taken into account. Thus, the surface irregularities increase θ, if θ at smooth surface exceeds 85°, and decrease θ, if θ is lower than 60°. Taking into account these conditions, one can conclude, that irregularity contribution due to rigid spheric spores should cause the contact angle θ to increase. However, on multiple change of contact angle no drop asymmetry was noted, that could be attributed to the influence of salient half-spheres on irregularities. That is why the irregularity correction of surface layer of filtered fungus conidia was not introduced.

DISCUSSION

Typical NBRIG spectra of the conidia of four type fungi are shown at Figure 1. The analysis of spectra showed, that the following absorption bands could be selected for identification of substances, contained in conidium wall: 1639–1641, 1550–1560, 1033–1100 and 831–835 cm^{-1}. These bands possess the following properties:

i) do not overlap, i.e. could be easily analyzed with respect to their position and intensity;

ii) show sufficiently large intensity differences from one type of fungus to another, i.e. are mostly applicable for quantitative analysis of substances, contained in conidium wall.

Absorption at 1639–1641 cm^{-1}. Proteins and glycanes[6] possess high absorption in this range. Protein absorption band, amid 1, includes valent oscillations of C═O bonds (~80%), valent oscillations of C—N bonds (~10%) and deformational oscillations of N—H bonds (~10%) of amide group. Conformation of protein molecule can be estimated by placement of amid 1 band. The absorptions at 1656, 1650 and 1637 cm^{-1} correspond to irregular structure of α-spyral and β-structure of protein molecules, respectively. The absorption in the range of 1639 cm^{-1} is also characteristic for β-glycanes, separated from the cell wall of fungi.[10,11]

Absorption at 1275 cm^{-1}. Absorption band is related to valent oscillations of phospholipid P═O bonds. One can discern phosphate groups, linked with proteins by hydrogen bonds, and the free ones. The first ones absorb at 1350–1220 cm^{-1}, the second ones—at 1300–1250 cm^{-1}. It can be noticed, that absorption at 1275

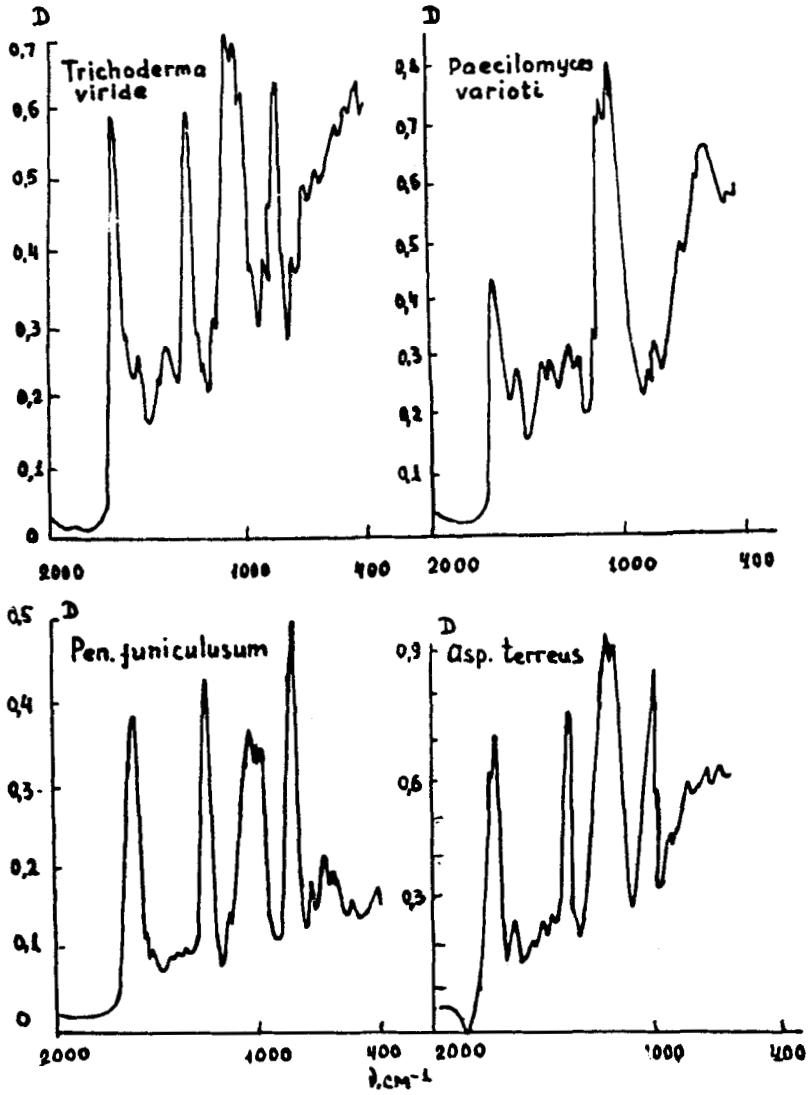

FIGURE 1 Typical IR spectra of outer fungal cell wall.

cm^{-1} is a characteristic for all types of fungi and indicates the presence of free phosphate groups.

Absorption at 1550–1540 cm^{-1} is stipulated by deformational oscillations of NH group (amid II), that are present in both proteins and chitin.

Absorption at 831 cm^{-1}, caused by deformational nonplanar oscillations of C—H group, is responsible for the presence of α-glycanes.[12] It was shown, that different α-glycanes: mannans, nigerans, separated from cell wall of fungi, exhibit absorption maxima in the range of 850–830 cm^{-1}.

Absorption at 1070–1050 cm^{-1},[4] associated with valent oscillations of CO group, is characteristic for glucanes and proteins.[4,5,10]

The base line was drawn through the lower points of peak bases for the calculation of intensities. Extinction coefficients were obtained from absorption spectra of individual substances of various concentrations for each substance at appropriate frequencies: chitin, proteins, phospholipids, glycanes.

Table I shows values of optical density for nine microscopic fungi.

The following approximations were made in the calculation of substance concentration in conidia walls:

i) Lambert-Buger-Bregg law is fulfilled for all substances:

$$C = \frac{D}{l\varepsilon}$$

where D = optical density; ε = extinction coefficient; l = beam penetration depth.

ii) Extinction coefficients are similar for each type of substance (chitin, protein, phospholipid, α-glycane) for all investigated microscopic fungi.

iii) Correlations of optical densities of different absorption bands for each microscopic fungus were used to avoid mistakes in obtaining the reflection spectra.

TABLE I

Optical densities of the main absorption bands of the wall of microscopic fungus conidia

Microscopic fungus	Optical density, rel.units				
ν, cm^{-1}	1639–1641	1550–1560	1275	1033–1100	831–835
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. *Trichoderma viride*	0.50±0.03	0.06±0.01	0.38±0.02	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. *Aspergillus flavus*	0.35±0.02	0.05±0.01	0.16±0.02	0.33±0.02	0.12±0.02
8. *Penicillium cyclopium*	0.28±0.02	0.05±0.01	0.17±0.03	0.58±0.03	0.17±0.02
9. *Paecilomyces variotii*	0.33±0.02	0.08±0.01	0.13±0.01	0.55±0.03	0.08±0.02

iv) The sum of mass parts of the main substances in conidium wall equals unity:

$$C_a + C_l + C_g = 1$$

where C_a = substances, containing amide group (proteins, chitin); C_l = phospholipids; C_g = glycanes.

Concentrations of phospholipids, α-glycanes and substances, containing amide groups, were calculated from correlations of optical densities of absorption bands, characterizing absorption of these substances. We used 1275 cm^{-1} for phospholipids, 831–835 cm^{-1}—for α-glycanes and 1550–1560—for amides.

$$\frac{D_g}{D_l} = AX, \qquad \frac{D_a}{D_l} = BY \tag{3}$$

where $A = l_g/l_l \cdot \varepsilon_g/\varepsilon_l$; $B = l_a/l_l \cdot \varepsilon_a/\varepsilon_l$; $X = C_g/C_l$; $Y = C_a/C_l$.

Using balance equation, we obtain:

$$C_l = \frac{1}{X + Y + 1}; \quad C_a = \frac{Y}{X + Y + 1}; \quad C_g = \frac{X}{X + Y + 1} \tag{4}$$

We experimentally estimated the following quantities for the above mentioned absorption bands:

$$\frac{l_g}{l_a} = 1.80; \quad \frac{\varepsilon_g}{\varepsilon_l} = 1.6 \pm 0.2; \quad \frac{l_a}{l_l} = 0.20; \quad \frac{\varepsilon_a}{\varepsilon_l} = 0.33 \pm 0.03$$

Calculated concentrations of phospholipids, α-glycanes and substances, containing amide groups (chitin, protein), are shown in Table II.

Absorption bands at 1639–1641 cm^{-1} and 1033–1100 cm^{-1} are complex, because they include absorptions of several groups:

$$D = \sum_i (\varepsilon_i C_i) \cdot l \tag{5}$$

The analysis of experimental data showed, that these bands consist of absorption bands of amides and α-glucanes.

According to our data, general content of main components in surface layer for nine types of microscopic fungi is: α-glycanes—0.05–0.25; phospholipids—0.26–0.72; amides—0.01–0.69.

The review of literature data regarding the composition of fungus walls shows different results. According to the data from Reference 10, the ratio of glycanes in external and internal walls of *Climitic* conidia is (3:2), i.e. glycanes are mostly concentrated at the external wall. According to results of Reference 12 external wall of several types of fungi contains 50% of proteins, 25% of phospholipids and glycanes up to ~15%. The other studies contain no information about the lipid content in the analysis of fungus wall composition. But they discuss glycanes and

TABLE II

Concentrations of phospholipids, α-glycanes and substances, containing amide groups, in walls of microscopic fungus conidia

No.	Microscopic fungus	Mass parts			Contact angle, grade
		amide	phospholipid	α-glucane	
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.	*Chaetomicin 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 variotii*	0.69	0.26	0.05	90

chitin ratios (6:1).[12] The differences in the analysis of fungus conidia wall should be attributed primarily to, different methods of sample preparation: disintegration, dissolution and use of, different biochemical reactions. Based on the analysis using NMR-spectroscopy of five types of gramm-positive bacteria, the authors[13] claim that peptide-glycanes are predominant compound in the cell wall of bacteria.

As it has been mentioned above, the contact angle θ is the parameter characterizing the fungus wall wetting by water. To determine the correlation between θ value and the wall composition for each type of fungus, the correlational equation was formulated, that reflects θ dependence on the sum of components with the respective coefficients of efficiency of each component (α, β, γ).

$$lg\theta_i = \alpha C_a + \beta C_l + \gamma C_g \qquad (6)$$

By solving the correlation equations for nine types of fungi, we obtained coefficient values, characterizing the contribution of each substance to the surface characteristics of fungus wall, stipulating water wetting, i.e. surface hydrophobia:

$$\alpha_a = 1.85 \pm 0.05; \quad \beta_l = 2.6 \pm 0.1; \quad \gamma_g = 0.75 \pm 0.05$$

The efficiency of external wall contribution to hydrophobia is defined predominantly by phospholipids, namely the coefficient β, whose value is 1.5–3.5 times higher than that of other coefficients.

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