Influence of the structural parameters on the biodegradation of poly-3-hydroxybutyrate and composites based on it

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SUMMARY
An investigation has been made of the probability of blends of poly-3-hydroxybutyrate (PHB) and ethylene–propylene rubber and 100% PHB being contaminated by micromycete species. Aspergillus flavus, Trichoderma viride, and Penicillium chrysogenum were most aggressive. The thermophysical characteristics after exposure to organic acids were determined.

Hundreds of millions of tonnes of artificial polymers are now being produced worldwide. At the end of the service life of polymeric materials, the critical question of their recycling arises, because, as is known, the decomposition period of many polymers under natural conditions is more than a decade long.

For more rapid ageing and breakdown of polymeric materials, different oxidants, chromophores, and biodegradable components are introduced into their matrix, which, on exposure to UV radiation, moisture, and microorganisms, promote fragmentation of the macromolecules. As the blended component, starch of different types and cellulose are often used, as readily available and relatively inexpensive types of raw material. However, in the past decade, in many countries, interest has grown in another biopolymer – poly-3-hydroxybutyrate (PHB), a linear polyester.

PHB exhibits high physicomechanical properties, similar to those of polypropylene (PP), compatibility with the human body, which is very important for medicine and pharmacology, and the ability to biodegrade. There have been studies in which the degradation of PHB and its copolymers in soil and on exposure to a weak alkaline solution has been investigated [1].

The aim of the present work was to investigate the possibility of developing micromycetes recommended by GOST 9.049-91 [2] for testing the mould resistance of polymeric materials, not only on specimens of 100% PHB obtained by casting from solution and pressing from a melt but also on blended composites with a synthetic polymer. Study of the effect of organic acids as model media is also important for a clearer idea of the mechanism of biodegradation.

As the second component, synthetic ethylene–propylene rubber (SKEP) was used. SKEP is an elastomer that is fairly widely used in industry. PHB–SKEP blends were studied by different methods, and the mechanical properties [3] and structural characteristics [4] were determined.

MATERIALS AND METHODS
In the work, use was made of PHB synthesised under anaerobic conditions with the aid of Ralstonia eutropha, and also SKEP of grade SO-059 (Dutral, Italy). Specimens of PHB and PHB–SKEP were produced by preliminary and principal mixing on a mill. Films of 100:0, 80:20, 70:30, and 50:50 composition (wt% PHB:SKEP) were pressed on a laboratory press. In the production of PHB films from solution, the solvent was chloroform.

The test strains were Aspergillus niger, Aspergillus
flavus, Penicillium purpurogenum, Penicillium brevicompactum, Penicillium chrysogenum, Penicillium cyclopium, and Trichoderma viride, provided by the Faculty of Mycology and Algology of the Lomonosov Moscow State University.

Wort agar was used as the nutrient. The possibility of uptake by the given micromycete species of different polymer composites was assessed using an optical microscope according to a five-rating scale: 0 – no germinated conidia or development of colonies; 1 – only small zones of mycelium can be seen in the form of individual spots, and no sporophyte reproduction; 2 – surface development of mycelium in the form of numerous spots, but no sporophyte reproduction; 3 – abundant growth of mycelium on the surface of the medium, and the start of sporophyte reproduction; 4 – on visual inspection, a continuous growth of mycelium and sporophyte reproduction are clearly evident; 5 – mycelium growing over the entire surface of the medium, with intense sporophyte reproduction. Monitoring was carried out for 3, 7, and 10 days.

The investigated composites were incubated for 12 days at $T = 26–27^\circ C$. Biomass accumulation by the biodegraders was determined by the gravimetric method. A Czapek medium was used as the control.

As the model medium for study of the mechanism of degradation of specimens, 5% solutions of citric and acetic acid were used. The experiment was conducted at $T = 20^\circ C$, after which the specimens were washed in distilled water and dried at $T = 20^\circ C$.

The thermophysical characteristics of the investigated composites were determined by differential scanning calorimetry (DSC). The scanning rate was 16 deg/min, the sample weight was varied from 8 to 15 mg, and calibration was done to indium with $T_m = 156.6^\circ C$. The degree of exposure of the specimens was assessed by means of IR spectroscopy on a Specord M 80 instrument with an accuracy of ±3 cm$^{-1}$ from the corresponding absorption bands in the IR spectrum: 3620 cm$^{-1}$ (isolated hydroxyl-containing groups) and 3420 cm$^{-1}$ (bound by hydrogen bonds) [5].

### RESULTS AND DISCUSSION

The dynamics of growth of the micromycete species under identical incubation conditions is different and is particularly clear by the time sporophyte reproduction begins.

As can be seen from Table 1, the start of growth and sporophyte reproduction are most rapid for *A. flavus*, *T. viride*, and *P. chrysogenum*, and a little less rapid for *A. niger*, and here the effect is most significant on the 50:50 composite. For practically all the strains used (with the exception of *P. purpurogenum*), the development of mycelium and sporophyte reproduction is observed. The active effect on the above composites can be attributed to structural defects and to considerable interphase interactions [6]. It was possible to assume that biopolymer PHB, obtained both from solution and from a melt, would be subjected to the most rapid effect of the micromycetes. However, it seems that, on account of the high degree of crystallinity and density, an effect of this kind is not observed. A similar effect is reported by Tsuji et al. [1], who show that, in soil, PHB undergoes degradation more slowly than its copolymer. After exposure for 7 days, *A. niger* was not inferior in activity to *A. flavus* and *P. chrysogenum*. As regards *P. purpurogenum*, the development of this test culture remained weak even after 10 days of experiment.

Biomass accumulation by the micromycete species and by the Czapek medium is presented in Figure 1.

The result is entirely as expected: the low-solubility substance (the polymer composite) prevents rapid change in the pH of the medium during the growth of mould. On a mineral Czapek medium, rapid biomass accumulation occurs. As regards the micromycete species, from Figure 1 it follows that differences in the magnitude of the biomass are not too significant for blended composites, and this quantity is lower only for PHB.

For clearer representation of the process of biodegradation of the composites investigated, citric and acetic acids were adopted as model media. For the case of 5% solutions of citric and acetic acids it was shown that specimens of 70:30 and 50:50 composition are most acid labile. Blended composites proved to be more susceptible to citric acid than to acetic acid. After exposure to the acids for 7 days, clouding of the

### Table 1. Assessment of the degree of intensity of sporophyte reproduction, 3 days, ratings

<table>
<thead>
<tr>
<th>PHB:SKEP specimen, wt%</th>
<th>A. niger</th>
<th>A. flavus</th>
<th>T. viride</th>
<th>P. brevi-compactum</th>
<th>P. cyclopium</th>
<th>P. chrysogenum</th>
<th>P. purpurogenum</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHB (melt)</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>PHB (solution)</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>80:20</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>70:30</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>50:50</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
50:50 specimens was observed, and a dip in the IR spectrum in the 3200–3800 cm\(^{-1}\) region. In this region, accumulation of hydroxyl-containing groups occurs. It must be pointed out that a similar effect was observed during oxygen absorption in an oxidising unit [7]. After washing and drying, these specimens became brittle, and the acid appeared to act on the –C–C– linkage in the main chain. Specimens of 100% PHB proved to be most resistant to the acids, even after 20 days at 20°C.

DSC was used to obtain the thermophysical characteristics for PHB and composites based on it before and after exposure to citric acid.

From Table 2 it can be seen that, after acid exposure, \(T_{\text{melt}}\) decreases from 5°C for PHB obtained from a melt to 10°C for the 50:50 composition, and the degree of crystallinity in the specimens investigated also decreases, which indicates the considerable effect of acid on the crystalline structure of PHB.

CONCLUSIONS

Thus, it can be concluded that blended composites based on PHB are subjected to the action of micromycetes, strains of which have been established and selected. By means of DSC and IR spectroscopy, the change in structure of the specimens after exposure to organic acids was shown, and it was established that 100% PHB is less susceptible than blended composites.

REFERENCES


Table 2. Thermophysical parameters of PHB:SKEP blends: initial blends and after action of citric acid (T = 20°C, 20 days)

<table>
<thead>
<tr>
<th>PHB:SKEP, wt%</th>
<th>(T_{1\text{melt}}), °C</th>
<th>(T_{2\text{melt}}), °C</th>
<th>(\alpha) of PHB before exposure to acid, %</th>
<th>(\alpha) of PHB after exposure to acid, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0 (melt)</td>
<td>174</td>
<td>169</td>
<td>47</td>
<td>44</td>
</tr>
<tr>
<td>100:0 (solution)</td>
<td>170</td>
<td>166</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>80:20</td>
<td>173</td>
<td>165</td>
<td>46</td>
<td>40</td>
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<td>70:30</td>
<td>172</td>
<td>163</td>
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<td>50:50</td>
<td>172</td>
<td>161</td>
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<td>43</td>
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