MACROMOLECULAR CHEMISTRY
AND POLYMERIC MATERIALS

Chemical Composition and Properties of Cultivated Wood-Rotting Fungi *Phanerochaete Sanguinea* and *Ganoderma Applanatum*


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Received July 20, 2000

Abstract—The fruit body composition of the cultivated wood rotting fungi *Phanerochaete sanguinea* 16–65, *Ganoderma applanatum* 4–94, and *Ganoderma applanatum* 40–90 and their sorption characteristics with respect to Cr(III) and Methylene Blue are studied.

The existing processes for cellulose bleaching with chlorine-containing reagents are necessarily accompanied by contamination of both the environment and the target product (bleached cellulose) with toxic chlorinated organic compounds (dioxins). In this connection searching for environmentally safe processes for cellulose bleaching using biological agents is a pressing problem. As known, it is possible to remove lignin from cellulose and thus increase its whiteness by direct treatment with white-rot fungus mycelium, pure hemicellulase (cleavage of ligno-carbohydrate bonds) and ligninase preparations (lignin degradation), and also with an enzymatic system from a mycelial culture. The latter case requires cultivation of lignin-degrading fungi having low cellulolytic activity. Efficient cellulose bleaching processes based on the use of lignin-degrading enzymes from various strains of wood-rotting fungi is under development at the St. Petersburg Academy of Forestry Engineering [1, 2]. Wastes from such processes represent fungal fruit bodies containing chitin, cellulose, melanin, and other components which can be used in various branches of industry and in medicine.

The goal of this work is to study the chemical composition of the cultivated wood-rotting fungi *Phanerochaete sanguinea* 16–65, *Ganoderma applanatum* 4–94, and *Ganoderma applanatum* 40–90 and to evaluate the sorption capacity of the fungal material and prospects for its utilization.

EXPERIMENTAL

Three fungal strains *Ph. sanguinea* 16–65, *G. applanatum* 4–94, and *G. applanatum* 40–90 were grown by surface cultivation in 500-ml Erlenmeyer flasks at 26°C in a culture medium (175 ml) developed by Gavrilova [3]. Its composition was as follows (g l\(^{-1}\)) : glucose 10.0, pentone 2.5, K\(_2\)HPO\(_4\) 0.4, MgSO\(_4\) 0.5, ZnSO\(_4\) 0.001, NaCl 0.3, FeSO\(_4\) 0.005, and CaCl\(_2\) 0.05. The culture medium was sterilized before use in an autoclave under pressure, cooled to room temperature, and inoculated with mycelial disks (0.5 mm) in Petri dishes on a pure culture agar. As the mycelium grew, the activity of oxidative enzymes in the culture medium was monitored. After reaching the peak activity the cultural liquors from different flasks, in which a given fungus was cultivated, were combined, filtered through a capron filter, and tested as cellulose bleaching agents.

The residual fungal fruit bodies were analyzed by successive extraction with hot water for 8 h, alcohol–benzene (1 : 2) mixture at bp for 8 h, and 6% NaOH at 100°C for 12 h. The residual insoluble chitin–glucan complex (CGC) was then hydrolyzed in concentrated HCl at 70°C for 7 h. The nonhydrolyzed fraction was additionally treated with a mixture of hydrogen peroxide and ammonia (1 : 10), and hydrolyzed again under the same conditions. The resulting hydrolyzates were combined and analyzed for glucose and glucosamine spectrophotometrically using color reactions of glucose with anthrone and of glucosamine with salicylaldehyde. The results are given in Tables 1 and 2.

† Deceased.
Infrared spectra were registered on a Specord 80/85 instrument (Carl Zeiss, Jena) using KBr technique.

Sorption experiments were conducted as follows. To a weighed portion of the sorbent (0.1 g recalculated to dry sample) we added 20 ml of a buffer solution and 10 ml of 0.01 M CrCl₃. After a lapse of time the sorbent was filtered off, and the residual Cr(III) was determined in the filtrate by chelometric titration [4]. Sorption of Methylene Blue was performed as follows. To a 0.3-g sample 20 ml of 0.5% Methylene Blue and 80 ml of distilled water were added. After a lapse of time the sorbent was filtered off, and the filtrate was analyzed spectrophotometrically for Methylene Blue. The sorption capacity for Esherichia coli was estimated from the amount of adsorbed Methylene Blue (0.016 g of adsorbed Methylene Blue corresponds to 176 × 10⁶ bacteria).

Table 1 shows that the highest moisture is observed for the fungus G. applanatum 4–94 and the least, for Ph. sanguinea 16–65, both growing on hardwood. Under natural conditions (in air, but not in aqueous medium as in this work) fruit bodies of these fungi are characterized by considerably lower moisture. The water-soluble fraction consisting of mineral salts and soluble hemicelluloses varies from strain to strain. The least content is found in G. applanatum 40–90 growing on softwood. The same strain contains the least amount of fats and resins representing the fraction soluble in the alcohol–benzene mixture. Hot aqueous NaOH dissolves proteins and melanin (partly). The percentage of this fraction is the highest (50–60%). The CGC content ranges from 12 to 20%, which is considerably lower than that in the mold fungus Aspergillus niger (50%), but comparable with that in the yeast Saccharomyces cerevisiae (12%) [5].

It was demonstrated by the IR spectra that the insoluble residue is represented by chitin–glucan complex. The spectra show the adsorption bands at 1203 and 1376 cm⁻¹ (CH and CH₂ groups) typical of cellulose and other glucans [6] and also at 1652, 1555, and 1310 cm⁻¹ (amide I, II, and III, respectively) [7] characteristic of chitin of various origins. The spectra are nearly identical with those obtained for CGC from mycelium of the fungus Aspergillus niger.

The elemental analysis data (Table 2) of the fungi after removal of the three soluble fractions demonstrate the presence of nitrogen, which is indicative of chitin.

Data on the composition of the insoluble fraction, obtained after it was totally hydrolyzed, confirmed the conclusion that this residue is represented by chitin–glucan complex. The hydrolyzed forms were demonstrated to be glucose, formed in cleavage of the glucan component of CGC, and glucosamine formed in hydrolysis of the chitin component to N-acetylglicosamine with simultaneous deacetylation. From these data we determined the composition of CGC isolated from the fungal fruit bodies (Table 3).

Table 3 shows that the chitin content in CGC is high in fruit bodies of all the strains studied, especially...
ly in G. applanatum 4–94 for which it is comparable with that in the wood rotting fungus Fomes fomentarius (71%) [5]. However, in contrast to the fungi studied, CGC from F. fomentarius additionally contains 10% intracellular melanin.

Such a high chitin content in CGC of the fungi studied and also the fact that their fruit bodies contain proteins suggest that these strains can be efficient ion exchangers for heavy metal ions. We studied the sorption properties of the fungi after treatment with hot water with an example of Cr(III). Sorption was performed over the pH range from 1.68 to 6.86. For all the fungi studied the maximal sorption in 24 h was observed at pH 6.86. The major fraction of Cr(III) was demonstrated to be sorbed in the first 10 min. The highest static exchange capacity (SEC) was shown by the strain G. applanatum 40–90 containing 61.1% proteins (Table 1) capable of fixing heavy metal ions.

Comparative analysis shows that CGC from the fungus Aspergillus niger demonstrates better sorption characteristics in strongly acidic solutions (depending on the composition. SEC for Cr(III) at pH 1.68 is 1.81–2.01 mg-equiv g\(^{-1}\) [8]). The strains studied in this work show better sorption from neutral solutions, and, although SEC obtained for these strains is lower, it should be taken into account that it refers not to CGC, but to fruit bodies only treated to remove the water-soluble fraction.

Finally, the fungal strains studied demonstrate high exchange capacity for Methylene Blue: recalculated to the sorbed amount of Esherichia coli it was found to be \(176 \times 10^6\), \(118 \times 10^6\), and \(317 \times 10^6\) for Ph. sanguinea 16–65, G. applanatum 4–94, and G. applanatum 40–90, respectively.

### CONCLUSIONS

(1) Fruit bodies of the cultivated fungi Ph. sanguinea 16–65, G. applanatum 4–94, and G. applanatum 40–90 contain up to 20% chitin–glucan complex, the relative content of chitin and glucan in CGC being dependent on the strain.

(2) The fungal strains studied show high sorption capacity for Cr(III), Methylene Blue, and Esherichia coli.

### ACKNOWLEDGMENTS

The authors are grateful to V.A. Solov’e and O.N. Malyshova for help in cultivating the wood-rotting fungi.

### REFERENCES