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APPLIED ELECTROCHEMISTRY AND CORROSION PROTECTION OF METALS

Electrochemical Recovery of Chitin–Glucan Complex from *Pleurotus ostreatus* Basidial Fungus and Properties of the Product

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Abstract—The chitin–glucan complex was recovered by an electrochemical procedure from the *Pleurotus Ostreatus* fungal mass. The chemical composition of the complex was determined, and its IR spectra were analyzed. The supramolecular organization of the isolated complex was determined by X-ray structural analysis. The sorption power of the complex toward Cu^{2+} and Fe^{3+} ions was evaluated.

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Crustacean shells are the major source of chitin today. Another large-tonnage source of chitin, fungi, for a long time was beyond researchers' attention, although chitin was found for the first time specifically in fungi in the beginning of the XIX century [1]. Wide occurrence of fungi in the nature and their high productivity make this source of chitin commercially significant, especially when using the mycelium of fungi applied in biotechnological processes for preparing organic acids, enzymes, and antibiotics. The location of these productions in industrial centers and large-tonnage production scale gave rise to the problem of utilization of mycelial wastes, and the stability of process conditions ensures standard characteristics of these wastes. These biotechnologies use lower fungi of the genera *Allomyces, Aspergillus, Penicillum, Mucor, Phicomyces*, etc. The most economically profitable way of mycelial waste utilization is their use for treating wastewater and concentrating nuclear wastes.

Along with mycelial fungi, of commercial interest is the large group of higher fungi (*Basidiomycota*) whose chitin content reaches 50 and even 65%, because they can be cultivated on wastes from wood processing, pulp-and-paper, and food industries. The fruiting bodies of *Basidiomycota*, both growing under natural conditions and cultivated in greenhouses, also contain chitin in an amount of 4–11% of dry weight of the fungal mass [2].

Chitin is present in cell walls of fungi, where it is bound with other polysaccharides, lipids, proteins, and microelements by ionic or hydrogen bonds. These complexes are more stable and specific than natural protein complexes of chitin in shells of invertebrates or in insect cuticles. Whereas proteins, lipids, and microelements can be relatively readily removed from the fungal mass by sequential extraction with organic solvents, dilute alkalis, and acids, it is impossible to separate chitin from the glucan moiety without polysaccharide degradation. Therefore, this component of the fungal mass was termed chitin–glucan ocmplex (CGC) and is being studied as a single whole.

It is known that CGC is not an individual substance. The ratio between chitin and glucan varies depending not only on the source but also on the conditions of cultivating the same fungus species. Nevertheless, more and more evidence appears for the covalent bonding between chitin and glucan, i.e., CGC cannot be considered as a mixture of two polysaccharides retained by labile hydrogen or ionic bonds. Most probably, CGC is a branched polysaccharide of variable composition in which the backbone is formed by chitin and pendant chains, by glucan (if the chitin

content is higher), or, vice versa, the backbone is formed by glucan and pendant chains, by chitin if glucan prevails (in *Saccsharomyces cerevisae* yeast). Some researchers believe that the chitin chain of CGC may contain, along with common chitin units, also "unusual" units in which the nitrogen atom is bonded not with the acetyl group but with a peptide bridge linking chitin and glucan chains of CGC [3, 4].

Studies on sorption properties of fungal chitin were initiated in 1980 by Muzzarelli [5]. In the subsequent period, it was shown that the sorption power of fungal chitin is higher than that of chitin of animal origin. This is due to different morphological structure of fungal chitin and its developed surface. Fungal chitin is organized in the form of microfibrils which form in fungus wall a voluminous network with a high active surface area, more than 1000 $m^2 g^{-1}$. The size of pores between the microfibrils is 50 E [6].

Up to now, CGC was recovered from fungal mass (mycelium) or finely divided fruiting bodies by an acid–alkaline or enzymatic procedure. The developed reagentless electrochemical procedure for chitin recovery from shell-containing raw materials (crabs, shrimps, gammarus) is very promising.

In this study, we examined the possibility of using an electrochemical procedure for CGC recovery from fungal raw materials. As a model we chose Pleurotus Ostreatus basidial fungus grown under greenhouse conditions, which allows control over the degree of fungus ripening and hence over its chemical composition.

EXPERIMENTAL

The mean moisture content of the *Pleurotus Ostreatus* fungal mass after defrosting was 89.67%. Composition of dry fungal mass, %: protein 43, glucans 48, chitin 8, with the remainder being lipids and microelements [7].

The CGC was isolated from the fungal mass by an electrochemical procedure in a diaphragmless electrolyzer with plane-parallel electrodes. The cathode was made of Cr18Ni19Ti stainless steel, and the anode, of platinized titanium. The casing was made of polypropylene sheet. The surface area of each electrode was 480 cm^2; the anolyte and catholyte volumes, 1 l each; the anode potential $E_a > 1200$ mV and the cathode potential $E_c < 950$ mV; pH ≥ 2.0 and pH ≤ 12.0, respectively; current density $i = 400$ A m^{-2}. The electrode spaces are separated by a fluoroplastic sulfonic cation-exchange membrane, which is chemically inert, heat-resistant, mechanically strong, and durable; it also has low ohmic resistance. As electrolyte we used a 1% NaCl solution at a solid-to-liquid ratio of 1 : 20.

Table 1. Composition of chitin–glucan complexes of *Pleurotus Ostreatus* fungus [7]

Content, %		Preparation procedure
glucans	chitin	
76–80	20–24	Chemical
82–85	15–18	Enzymatic

Fruiting bodies were finely divided, soaked in a 1% NaCl solution for partial release of proteins, washed, and squeezed. Deproteinization was performed in the cathode chamber of the electrolyzer, with intermittent measurements of pH of the catholyte until pH 12.3 was attained. Then the anolyte and catholyte were removed, and the raw material was squeezed and subjected to repeated deproteinization under the same conditions. Demineralization was done in the anode chamber, with the treatment performed until pH 1.4 was attained. The resulting CGC was allowed to stand for 24 h in the anolyte, after which it was washed to a neutral pH value. The washing quality was checked by the absence of chloride ions in the wash water. The product was dried in air at room temperature.

The product was analyzed for the total nitrogen content by the Kjeldahl method with a Perstorp Analytical system and for the ash content. Also, its IR spectra were taken (Bruker IFS-88 spectrometer, Germany), and X-ray diffraction analysis was performed (DRON-2 diffractometer, Ni-filtered CuK_α radiation). Sorption of Cu^{2+} ions from a 0.01 M copper acetate solution was studied using the technique of separate samples at pH 1.8 and 6.15. The concentration of copper ions was determined by complexometric titration. Sorption of Fe^{3+} ions from a sample of a cutting fluid (CF) was studied with a Tecator flow injection colorimeter allowing determination of trace concentrations of iron in a sample.

Chemical and enzymatic methods for the recovery of chitin–glucan complexes are known. The chemical procedure involves sequential treatment with organic solvents for the recovery of lipids, with acid solutions for demineralization, and with alkali solutions for the extraction of proteins. The process is accompanied by degradation of the biopolymer, mainly at the expense of cleavage of glucan chains. As a result, the chitin content

Table 2. Results of analysis of the electrochemically prepared chitin–glucan complex

Sample	Parameters of electrochemical treatment						Fungus biomass		Isolated CGC	
	deproteinization			demineralization			N_{tot}	ash	N_{tot}	ash
	τ, min	*T*, °C	pH	τ, min	*T*, °C	pH	%			
1_1	9	39	12.3							
1_1	10	41	12.3	50	62	1.7	4.04	7.87	3.56	1.19
2_1	10	41	12.5							
2_2	10	32	12.6	25	56	1.4	3.83	5.03	1.64	0.42

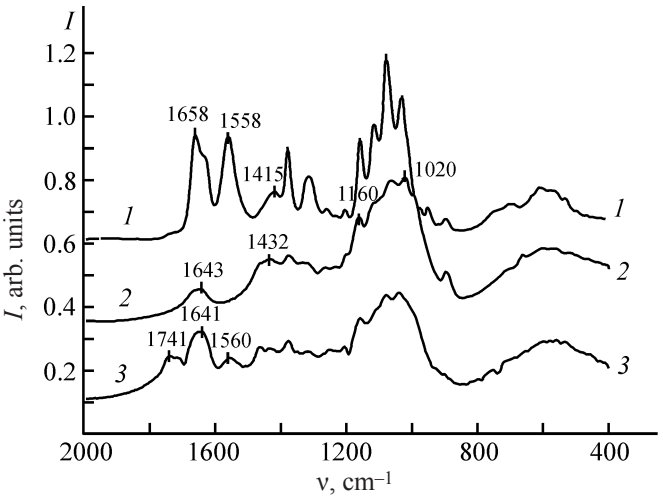

Fig. 1. IR absorption spectra of (*1*) crab chitin, (*2*) cotton cellulose, and (*3*) chitin–glucan complex of Pleurotus Ostreatus. (I) Intensity and (ν) wavenumber.

of CGC increases and can reach 85–90% [8].

The enzymatic procedure of CGC recovery using Prolive complexes of proteolytic enzymes, protease, β-glucosidases, and cellulase ensures preservation of the glucan component [7].

The resulting CGC is analyzed for the content of D-glucosamine, acetyl groups, and neutral sugars. The composition of the *Pleurotus Ostreatus* CGC isolated by chemical and enzymatic procedures is given in Table 1.

The electrochemical process for chitin recovery from shell-containing raw materials and its implementation are described in detail in [9].

The electrochemical procedure for chitin recovery has a number of significant advantages over the chemical and enzymatic procedures, one of which is the possibility of preparing a wide range of chitin derivatives and controlling the processing parameters depending on the process requirements.

To recover CGC from a fungal raw material, the electrochemical procedure was applied for the first time. The use of the mode developed for processing of crab shells showed that even twofold treatment of the fungal raw material in the cathode chamber did not ensure complete removal of proteins (Table 2, sample 1_2): The N_{tot} content in the product decreased by only 0.5% relative to the raw material. Dimeralization was more successful: the ash content decreased by a factor of almost 6. Therefore, the processing mode was changed. The fungal raw material was subjected to more thorough dispersion and preliminary soaking in a 1% NaCl solution to remove water-soluble proteins. After repeated treatment in the cathode chamber, the catholyte temperature was raised to 85°C, and the product was kept in the catholyte for 30 min. Simultaneously the time of treatment in the anode chamber was decreased by half. These modified conditions allowed preparation of a CGC with a low degree of mineralization and satisfactory degree of deproteinization (Table 2, sample 2_2): The N_{tot} content corresponds to that in CGC samples isolated from other sources [10]. Hence, the electrochemical procedure is applicable to CGC recovery from a fungal raw material.

The composition of Pleurotus Ostreatus CGC was confirmed by the IR spectra (Fig. 1). Because the chitin–glucan complex consists of chitin and glucan and the monomeric unit of glucan, glucose, is the same as in cellulose, we also present for comparison the IR spectra of chitin and cellulose.

The most informative range in the IR spectra of these polymers is the range from 400 to 1800 cm^{-1}. In this part of the CGC spectrum, there are characteristic absorption bands typical of both chitin and cellulose: 1030 cm^{-1}, bending vibrations of glycoside bond; 1430, primary hydroxy groups at C^6 atoms in all the three polymers.

Chitin and chitin–glucan complex differ from cellulose in that they contain an amide group at the C^2 atom. This difference is manifested in the spectra of chitin and CGC in the form of the Amide I (1660–1650 cm^{-1}) and Amide II (1560–1550 cm^{-1}) absorption bands. A characteristic feature of the electrochemically isolated *Pleurotus Ostreatus* CGC is an absorption band at 1710–1750 cm^{-1} corresponding to carbonyl groups. These groups appear in CGC owing to oxidation of hydroxy groups in chitin and glucan constituents of CGC, and also to cleavage of glucoside bonds with the formation of terminal aldehyde groups. These groups in CGC are formed in the anode chamber of the electrolyzer, where highly active oxidants are generated.

X-ray diffraction analysis showed (Fig. 2) that the structural organization of the electrochemically isolated CGC is similar to that of chemically isolated CGC samples. The X-ray diffraction pattern of CGC contains two diffuse reflections in the range of angles 2θ 9°–11° and 18°–22°. In the diffraction pattern of chitin, in these regions there are usually well-defined reflections associated with the intermolecular packing of chains (2θ 9° and 20°), and also reflections indicative of the long-range ordering of the polymer chains in planes 001 (2θ 9°), 002 (2θ 17°30′), 003 (2θ 26°30′), and 004 (2θ 35°), with the latter two reflections being very strong. The diffraction pattern of *Pleurotus Ostreatus* CGC shows that the intermolecular packing of chitin–glucan chains does not form well-defined crystalline structures. The packing character is mesomorphic, i.e., intermediate between crystalline and amorphous. The glucan component does not form an intrinsic structure but prevents highly ordered packing of chitin chains. The diffraction pattern of *Fomes Fomenfarius* CGC isolated by an acid–alkaline procedure contains a well-defined strong reflection at 2θ 20°, suggesting more ordered supramolecular organization. Additional amorphization of *Pleurotus Ostreatus* CGC is caused by oxidative degradation of CGC in the course of electrochemical recovery, leading to a decrease in the molecular weight and to formation of new functional groups.

To determine the sorption characteristics of *Pleurotus Ostreatus* CGC, we studied the kinetics of sorption of copper ions in relation to pH of solution and estimated the total exchange capacity (TEC) of CGC.

As seen from Table 3, the Pleurotus Ostreatus chitin–glucan complex isolated by an electrochemical procedure exhibits better sorption properties in a medium close to neutral. At both pH values, intense sorption is followed by desorption which occurs at pH 1.8 in 30 min and at pH 6.15 in 60 min. The desorption is also observed with other chitin-containing sorbents. A decrease in the amount of sorbed ions may be due to realization of two sorption mechanisms: chelating complexation and physical sorption. Branched CGC has an amorphous supramolecular organization favoring formation of pores and voids in amorphous regions, which can retain metal ions. With an increase in the sorption time, a part of

Table 3. Sorption of ions by *Pleurotus Ostreatus* CGC at various pH values

Sorption time, min	pH 1.8		pH 6.15	
	Sorbed Cu, mg g^{-1} sorbent	TEC, mg-equiv g^{-1}	Sorbed Cu, mg g^{-1} sorbent	TEC, mg-equiv g^{-1}
10	1.69		6.23	
30	1.04		8.29	
60	0.62		6.69	
120	–		6.54	
180	–		6.12	
1440	1.26	0.04	7.09	0.26

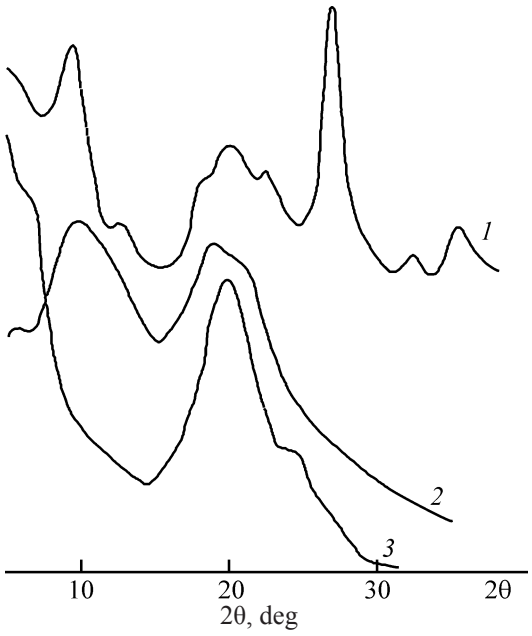

Fig. 2. X-ray diffraction patterns of (*1*) crab chitin, (*2*) *Pleurotus Ostreatus* chitin–glucan complex, and (*3*) Fomes Fomenfarius fungus chitin–glucan complex. (2θ) Bragg angle.

Table 4. Sorption of ions from cutting fluid by *Pleurotus Ostreatus* CGC

Time, min	Content of Fe^{3+} ions in CF, g l^{-1}	Amount of sorbed Fe^{3+} ions, %
0	0.150	0
15	0.053	65
30	0.030	78
60	0.047	69

physically sorbed ions undergo desorption, whereas the chelating sorption mechanism leads to the formation of stable complexes.

Practical studies on treatment of industrial wastewaters to remove heavy metals using chitin–glucan complexes are few. In the majority of studies, sorbents prepared from fungus cell walls are used as powder suitable for filling experimental filtration columns. Attempts were also made to improve the service characteristics of such sorbents. Selective membranes were made of chitosan [11, 12], of a material similar to filter paper and prepared directly from fungal hyphae, or of nonwoven materials with additions of other fibers. There are also data on attempts to use native fungal mycelium for recovery of heavy metals. In many cases, this material appeared to be fairly effective in recovery of Cr, Au, Co, Ag, As, Pt, and other ions [13].

The main disadvantage of lower fungi is poor mechanical strength of their mycelial fibers. Furthermore, in the course of drying the fibers stick to each other. In the dry state, such materials become brittle, readily crack, and raise dust. In the wet state, they noticeably swell and plug columns [14]. Despite ample and diverse data on sorption of heavy metals with chitin, chitosan, and their derivatives, there are still no operating installations, at least semicommercial, for wastewater treatment with these materials. There are apparently two reasons for this fact: the available sorbents are too expensive, or their process characteristics are unacceptable [15].

The *Pleurotus Ostreatus* chitin–glucan complex that we obtained well competes in characteristics of sorption of copper ions with chitosan prepared from a shell-containing raw material [16] and with chitosan prepared from Chitinel [17].

To evaluate the suitability of the chitin–glucan sorbent prepared for wastewater treatment, we studied experimentally the sorption of Fe(III) ions from a sample of cutting fluid taken from Moskabel' enterprise (Moscow). This recyclable liquid is saturated with sodium sulfate, has pH 7, and contains impurities of Fe^{3+} ions in an amount of 0.15 g l^{-1}.

Our studies showed that, at pH close to neutral, the maximal sorption is observed in 1 h. Therefore, the sorption kinetics in evaluation of the degree of cutting fluid purification was monitored during a period of 60 min.

As seen from Table 4, the major fraction of iron ions (78%) is sorbed already in the first 30 min, which is followed by desorption. Hence, for more efficient recovery of Fe^{3+}, the sorption time can be reduced. Thus, CGC electrochemically recovered from *Pleurotus Ostreatus* shows promise for treatment of cutting fluids from metal-working plants.

As compared to analogous sorbents (chitin–glucan complexes of other fungi, or *Pleurotus Ostreatus* CGC recovered by traditional acid–alkaline and enzymatic procedures), the sorbent we prepared is cheaper because of the use of the electrochemical technology (compared to the enzymatic procedure), and its production is environmentally cleaner (compared to the acid–alkaline procedure).

CONCLUSIONS

(1) Conditions were determined for electrochemical recovery of chitin–glucan complex from *Pleurotus Ostreatus* fungal mass, ensuring high degrees of demineralization and deproteinization.

(2) The electrochemically recovered chitin–glucan complex, in contrast to that recovered chemically, contains carbonyl groups, is environmentally cleaner, and exhibits sorption activity toward Cu^{2+} and Fe^{3+} ions.

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