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Isolation of Chitin-Containing Complexes from the Thallus of the Lichen Species *Peltigera aphthosa*

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Abstract—Chitin-containing complexes were obtained from the thalli of the foliose lichen *Peltigera aphthosa* by supercritical fluid extraction (SCFE) and acid–base hydrolysis. In an active designed experiment, the optimal SCFE conditions were determined, which made it possible to obtain a complex with a yield of 96% and a sorption capacity for methylene blue of 125 mg/g. The polyampholytic nature of the resulting sorbent complexes is shown, and their morphology and chemical composition are characterized. The processing of lichen raw materials to produce chitin-containing complexes by SCFE is shown to be more efficient than acid–base hydrolysis.

Keywords: supercritical fluid extraction, carbon dioxide, acid–base hydrolysis, epigean lichen, *Peltigera aphthosa*, chitin-containing complex

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INTRODUCTION

A new class of sorbents consisting entirely or partially of substances of biogenic origin (biosorbents) has recently been widely studied worldwide. The most widely used materials are polymers based on chitin (chitosan) with an extensive raw material base (shells of marine crustaceans, fungi, dead bees, etc.), which is constantly expanding by including new alternative sources. The unique properties of chitin (chitosan) are its high sorption capacity, low ash content, biodegradability to substances safe for wildlife, and the possibility of obtaining sorbents consisting of particles with the given geometry and a large surface.

The largest amount of chitin is contained in the integumentary shells of red king crab and krill [1, 2]. The isolation of chitin from the shell of crustaceans is accompanied by the formation of a large amount of waste, which leads to environmental pollution and is considered a serious disadvantage. An alternative to solve these problems is the production of chitin from various classes of fungi, which is potentially more environmentally friendly [3]. The properties (molecular weight, solubility, degree of crystallinity) and the structure of the isolated chitin can vary significantly depending on the source of the raw material and the method of obtaining the polymer.

The chitin content in the cell walls of higher fungi ranges from 5.2 to 80% and depends on the species of fungus, the age of the fungal cell, and the conditions of its growth. For lower fungi, the chitin content is 0.2–26.2% and is maximum in aspergillus (20–22%) [4–6].

A special group of fungi is lichenized ascomycetes (lichens), the cell walls of which are also formed of chitin microfibrils. This is a group of living organisms, which comprises more than 20000 species. In lichens, a fungus (mycobiont) and a unicellular green algae (phytobiont) form a single organism, which can additionally involve cyanobacteria (cyanobiont) [7, 8]. The cell walls of these symbiotic associations consist of mycobiont for 90–98% [9–11].

In the cell walls of higher fungi and some lichen species, chitin is present in the form of a chitin–glucan complex, in which it is bound to 1,3- and 1,6-β-glucans and performs protective functions [12]. In some species of fungi and lichens, the melanin pigment is embedded in the structure of a chitin-containing complex (CCC). Melanin is an important biologically active component of the cell wall: it exhibits antioxidant, hepatoprotective, and antimutagenic properties and it can absorb heavy metals and organic pollutants and neutralize lipid peroxidation products [13].

There are only a few studies of chitin and CCCs in lichens [9, 14, 15] and they are aimed at determining the physicochemical properties of lichen chitin and comparing them with the properties of chitins obtained from other sources.

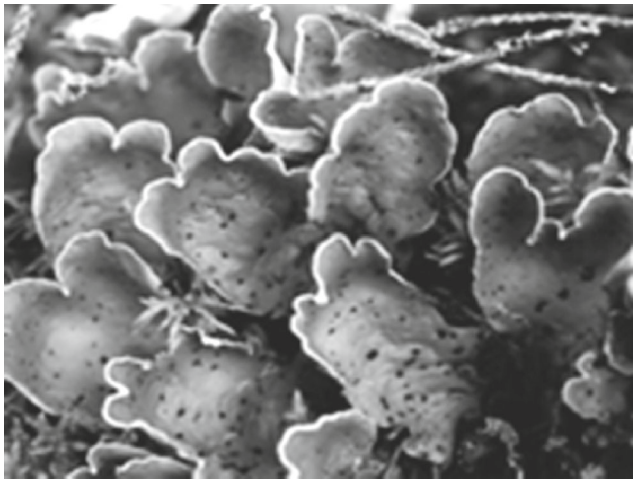

Fig. 1. Appearance of the thallus of the lichen *P. aphthosa*.

The isolation of CCCs from ascomycetes is typically performed by successive hydrolytic treatment with boiling water with the addition of EDTA (ethylenediaminetetraacetic acid), ethanol with alkali, and hydrochloric acid. This method is also applicable to micromycetes [16, 17]. When isolating CCCs from higher basidiomycetes, multistage methods of sequential acid–base hydrolysis of fungal biomass are used, including the stage of depigmentation:

$$NaOH \rightarrow HCl \rightarrow H_2O_2 \rightarrow NaOH, \quad (1)$$

$$HCl \rightarrow NaOH \rightarrow HCl \rightarrow H_2O_2 \rightarrow NaOH, \quad (2)$$

$$NaOH \rightarrow HCl \rightarrow H_2O_2 \rightarrow NaOH \rightarrow HCl. \quad (3)$$

It was determined that four-stage processing is sufficient to obtain high-quality chitin [18].

Together with the conventional methods for obtaining CCCs from various species of fungi, there is an extraction method, which is based on the extraction of low-molecular-weight components to obtain CCCs in the form of a meal. One of the modern extraction methods is supercritical fluid extraction (SCFE), which makes it possible to both isolate CCCs and simultaneously extract slightly modified extractive biologically active substances [19–24].

The advantage of using carbon dioxide as an extractant in SCFE is its low cost and the possibility of recycling [25]. Although production of biologically active substances from lichens was studied in numerous works [19–22], there are no published data on the use of SCFE for the isolation of chitin and its complexes.

Currently, technological processes, including SCFE, are often optimized by methods of an active designed multifactorial experiment with constructing second-order uniform designs, which make it possible to build a quadratic regression model in the form of a second-degree polynomial that takes into account the effect of at least three factors [22, 24, 26–32].

The aim of this study is to compare the morphological and physicochemical properties of CCCs isolated by acid–base hydrolysis and supercritical fluid extraction from the lichen *P. aphthosa*.

EXPERIMENTAL

The raw material for the isolation of CCCs was epigeic foliose lichen of the species *P. aphthosa* (Fig. 1), which was gathered in the Kholmogory district of Arkhangelsk region. It belongs to three-component lichens: the function of carbon assimilation in it is mainly performed by green algae, and cyanobacteria are contained in specific structures called cephalodia and fix atmospheric nitrogen.

Lichen thalli were cleaned to remove impurities (bark particles) in the laboratory and dried in air in the absence of direct sunlight. The moisture content (6.2–0.2%) and ash content (2.1–0.1%) of the thalli were determined according to the standard procedures [33]. Before the isolation of CCC, the lichen thalli were preliminarily ground in an LN-201 laboratory mill. For the study, a fraction of 0.2–0.5 mm in size was used, which was 90 wt % of the ground sample.

CCCs were isolated from the lichen *P. aphthosa* by supercritical fluid extraction and acid-base hydrolysis (ABH). SCFE was carried out on a SCFE-5000 unit (Waters, United States) equipped with a 100 mL extraction cell; the weight of the sample of the raw material was 100 g of absolutely dry raw material.

To mathematically describe SCFE of the lichen *P. aphthosa* and determine the optimal parameters of the process, a designed experiment was carried out with the construction of a second-order central composite rotatable uniform design [34]. The following parameters were chosen as the main levels of processing parameters: pressure (X) 25.0 MPa, temperature (Y) 60°C, and specific mass flow rate of extractant (SMFRE) (Z) 35 at an extractant flow rate of 50 g/min; the ranges of variation of parameters were 6.0 MPa, 15°C, and 10, respectively. The distance between the central and star points of the design was 1.682. Table 1 presents the levels of the factors.

Table 2 presents the design matrix in coded and natural form and the obtained results: the yield of CCC (%) and its sorption capacity (SC) for methylene blue (MB) (mg/g).

CCCs were isolated from the lichen thalli by the ABH method [35] from which the stage of depigmentation was excluded because this stage is performed to remove the melanin pigment having high sorption capacity for organic pollutants and heavy metals [36]. Figure 2 shows the process flow diagram of the isolation of CCCs from the thallus of the lichen *P. aphthosa*.

The yield of the final product (as a weight percentage of the initial raw material, taking into account the

Table 1. Levels of factors in optimization of the isolation of a chitin-containing complex (CCC) from the lichen *P. aphthosa* by SCFE

Variable factors	Notation of factor	Levels of factors and variation intervals					
		step of variation	range of variation				
			−1.682 (−α)	−1	0	1	1.682 (α)
Pressure, MPa	X	10.0	8.2	15.0	25.0	35.0	41.8
Temperature, °C	Y	20	26	40	60	80	93.5
SMFRE	Z	15	9.8	20	35	50	60.2

Table 2. Experimental design matrix in natural form

No. of exp.	Level of factor			Yield of CCC, *W*, %	Sorption capacity, SC, mg/g
	X, MPa	Y, °C	Z		
1	15.0	40	20	100	76.4
2	35.0	40	20	99.5	82.1
3	15.0	80	20	100	38.2
4	35.0	80	20	95.6	127.4
5	15.0	40	50	100	72.2
6	35.0	40	50	98.2	117.4
7	15.0	80	50	100	70.8
8	35.0	80	50	98.8	116.0
9	8.2	60	35	100	72.2
10	41.8	60	35	99.4	82.1
11	25.0	26	35	100	97.6
12	25.0	93.5	35	100	106.1
13	25.0	60	9.8	100	66.5
14	25.0	60	60.2	94.5	131.6
15	25.0	60	35	96.4	124.5
16	25.0	60	35	100	101.9
17	25.0	60	35	99.6	113.2
18	25.0	60	35	100	102.2
19	25.0	60	35	100	109.0
20	25.0	60	35	98.8	116.0

moisture content) was determined by weighing the sample after drying in a Lyovapor L-200 freeze dryer (Buchi, Switzerland).

Elemental analysis (C, H, N) of the lichen thallus and CCC was performed by burning the sample in a flow of pure oxygen at an oven temperature of 1200°C in a combustion tube (24 mm i.d.) with an Elementar Vario MICRO cube elemental composition analyzer (Elementar Analysensysteme GmbH, Germany). The arithmetic mean of the results in triplicate was taken as the final result and the discrepancy between the results did not exceed 10% of the arithmetic mean.

The content of extractives in the samples was determined by exhaustive extraction with a mixture of solvents (1 : 1 hexane–chloroform mixture) in a Soxhlet apparatus [37].

The melanin content was found by the method of depigmentation with an ammonia solution in the presence of hydrogen peroxide [35, 38]. The degree of depigmentation was estimated by changing the optical density of the solutions with a UV-1800 spectrophotometer (Shimadzu, Japan) at a wavelength of 450 nm. The melanin content was determined from the weight loss of the sample during depigmentation.

The content of proteins in the samples was determined by the Lowry method [38]; crystalline albumin (Sigma-Aldrich) was used as the standard.

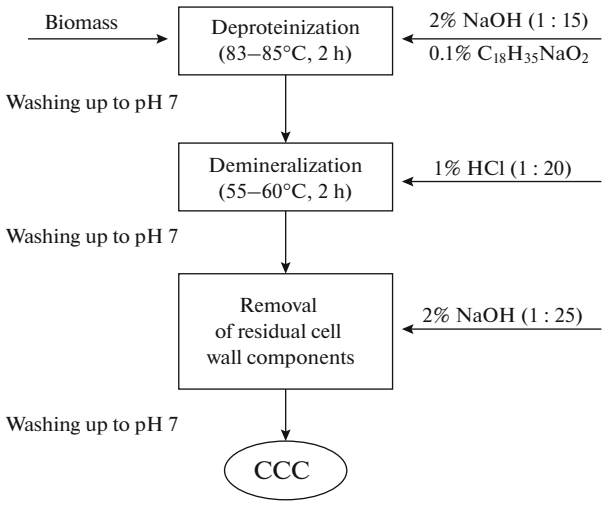

Fig. 2. Process flow diagram of the isolation of a chitin-containing complex from *P. aphthosa* biomass by acid–base hydrolysis.

The content of chitin was estimated by visible spectroscopy by the formation of chromogens between the products of acid hydrolysis (glucosamine) and an alkaline solution of acetylacetone and Ehrlich's reagent (4-dimethylaminobenzaldehyde) [40].

The IR spectroscopic studies of the chitin and CCC samples compacted into tablets with KBr were performed with an IRAffinity-1 IR Fourier spectrometer (Shimadzu, Japan; scanning range 4000–400 cm^{-1}, number of scans 50, resolution 4 cm^{-1}).

Images of the freeze-dried samples of chitin and CCC were taken with a SEM Sigma VP scanning electron microscope (Zeiss, Germany). To increase the contrast of the images, a platinum–palladium coating up to 5 nm thick was applied to the surface of the samples using a Q150TES turbomolecular pumped coater (QUORUM).

The X-ray powder diffraction patterns of chitin and CCC samples preground with a Retsch PM100 planetary ball mill were recorded with an XRD-7000S diffractometer (Shimadzu, Japan) equipped with an attachment for sample rotation and a polycapillary optical system. The X-ray tube operating parameters were the following: accelerating voltage, 50 kV; current, 30 mA, material of target, Cu; 2θ range, 10°–70°; scanning speed, 0.5 deg/min; and scanning step, 0.02 deg.

The sorption capacity of the CCCs was estimated by a static method [41] for the methylene blue (MB) and Congo red (CR) dyes, which are models of endotoxins and organic pollutants of medium and low molecular weights [42–47].

The experimental results were presented as the arithmetic mean and its absolute error in triplicate. To determine the statistical relationship between the parameters, the Student's *t*-test was used at a confidence level of $P = 95\%$.

RESULTS AND DISCUSSION

Twenty experiments were carried out to determine the optimal parameters of the isolation of the chitin-containing complex by SCFE from the thalli of the lichen *P. aphthosa* (Table 2). The yield of CCC ranged from 94.5 to 100%; and the SC of the obtained material for MS, from 38 to 132 mg/g. At the center of the design, six experiments were carried out, the analysis of the results of which showed their high convergence: the deviation from the average yield did not exceed 2.5 rel %. In the designed experiment, the minimum yield of CCC, corresponding to the most complete extraction of extractable substances, was detected at an SMFRE of 60 and zero levels of pressure and extraction temperature factors (i.e., at 25.0 MPa and 60°C) and was 94.5% (Table 1). The scatter of the values of the sorption capacity of the materials at the center of the design turned out to be an order of magnitude higher, and the deviation from the mean reached 12%. The material with the highest sorption capacity was obtained under the conditions of the maximum extraction of extractable substances.

Mathematical processing of the results using the MS Excel software gave the following regression equations:

$$W = 99.9 - 4.1x - 3.3z - 2.9xy + 0.3yz + 3.6x^2 + 1.2y^2 + 1.5x^2, \quad (1)$$

$$\mathrm{CE} = 111.1 + 14.8x + 11.8z + 10.4xy - 1.2yz - 12.8x^2 - 4.1y^2 - 5.1z^2, \quad (2)$$

where $x = (X - 250)/100$, $y = (Y - 60)/20$, and $z = (Z - 35)/15$. These equations adequately (with sufficient accuracy) describe the dependences of the yield of CCC (W, %) and its sorption capacity (SC) for MB (mg/g) on the conditions of the extraction and contain only significant coefficients. The equations were tested for adequacy by the Fisher criterion at a significance level of 0.1.

The multiple correlation coefficients of the experimental and calculated values of the yield and SC were 0.90 and 0.85, respectively, at relative model errors σ of 2.7 and 12.4%, respectively.

An analysis of the obtained regression equations determined the optimal values of the process factors corresponding to the maximum possible removal of extractable substances (minimum yield of CCC) and the maximum SC of the produced material. The pressure, temperature, SMFRE in the optimal experiment were 35.0 MPa, 80°C, and 50, respectively, for both optimization parameters. The expected yield of CCC was 96.3% with a sorption capacity of 125.1 mg/g. In the confirmatory experiment, a CCC sample was

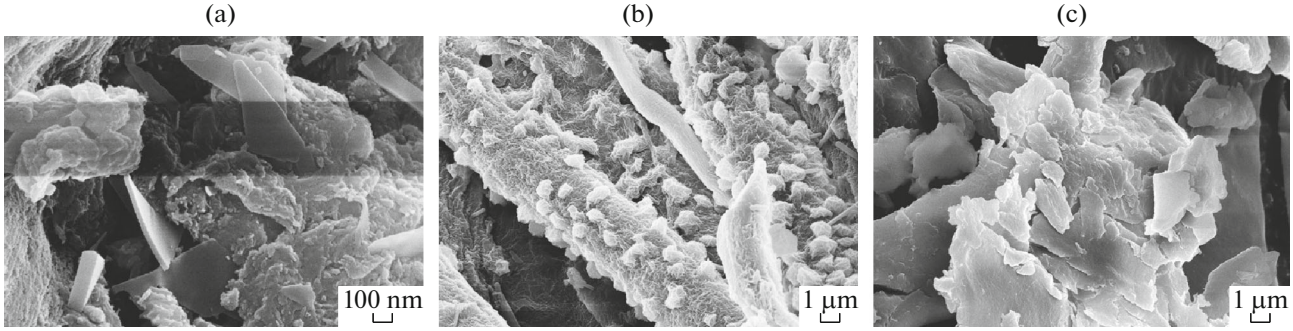

Fig. 3. Ultramicroscopic structure of the surface of (a) crab chitin and the chitin-containing complexes (b) CCC_{SCFE} and (c) CCC_{ABH}.

obtained with a yield of 96.0% at the SC of 125 mg/g. The relative model error was less than 1%.

The isolation of CCCs by ABH comprised two main stages—deproteinization and demineralization—and the final stage of alkaline treatment (Fig. 2). The treatments were carried out by the infusion method; after each stage, the material was washed with distilled water until the medium was neutral. The deproteinization was performed for 2 h with an aqueous solution containing 2% sodium hydroxide and 0.1% sodium stearate (surfactant) at a temperature of 83–85°C and an SMFRE of 15. The demineralization was conducted for 2 h with a 1% aqueous solution of hydrochloric acid at a temperature of 55 to 60°C and an SMFRE of 20. The final alkaline treatment was carried out for 2 h with a 2% aqueous solution of sodium hydroxide at a temperature of 83–85°C and an SMFRE of 25. The yield of CCC was 24.9–0.2%.

The CCCs isolated by SCFE and ABH (CCC_{SCFE} and CCC_{ABH}, respectively) were finely divided black powders, which did not differ from each other outwardly. Figure 3 shows the scanning electron microscopy images of the surface of the obtained CCCs in comparison with crab chitin.

CCC_{SCFE} largely retains the structure of the cell wall of the initial lichen (elements of the structure are clearly visible in Fig. 3b). CCC_{ABH} has a more altered structure similar to the structure of crab chitin (Figs. 3a, 3c) because of its preparation by similar acid–base treatment methods. On the surface of crab chitin, CCC_{SCFE}, and CCC_{ABH}, there are similar crystal structures, but most of the surface consists of amorphous regions.

Table 3 compares the compositions of the CCCs isolated by SCFE and ABH and the initial lichen thallus.

The supercritical fluid extraction removes almost all (97.5%) of the extractives, whereas components such as chitin, melanin, protein, and minerals (ash) are practically not removed and remain in the structure of CCC_{SCFE}. This is caused by the almost complete insolubility of these components in supercritical carbon dioxide. By contrast, the severe acid–base treatment removes significant fractions of chitin (23%), melanin (65.4%), protein (98.5%), and minerals (58.5%). The removal of protein and minerals by ABH is a beneficial result because their content in the enterosorbent is regulated [42, 48] and should not exceed 2 and 3%, respectively. Thus, CCC_{SCFE} does not meet the requirements for enterosorbents in terms of the content of residual protein and minerals.

Table 3. Component and elemental compositions of the thallus of the lichen *P. aphthosa* and chitin-containing complexes

Component	Component content, %/fraction of component removed during treatment, rel %		
	lichen thallus	CCC_{SCFE}	CCC_{ABH}
N	3.6 ± 0.1	3.9 ± 0.1	1.8 ± 0.1
C	48.9 ± 2.0	59.9 ± 0.3	57.0 ± 0.3
H	6.5 ± 0.3	5.8 ± 0.2	5.4 ± 0.2
Ash (minerals)	2.1 ± 0.1	2.8 ± 0.4/0	3.5 ± 0.3/58.5
Chitin	1.02 ± 0.09	1.20 ± 0.10/0	3.15 ± 0.09/23.1
Melanin	9.0 ± 0.6	9.4 ± 0.4/0	12.5 ± 1.0/65.4
Protein	7.5 ± 0.9	7.8 ± 0.1/0.35	0.45 ± 0.06/98.5
Extractives	2.9 ± 0.9	<0.1/>97.5	0.9 ± 0.2/92.3

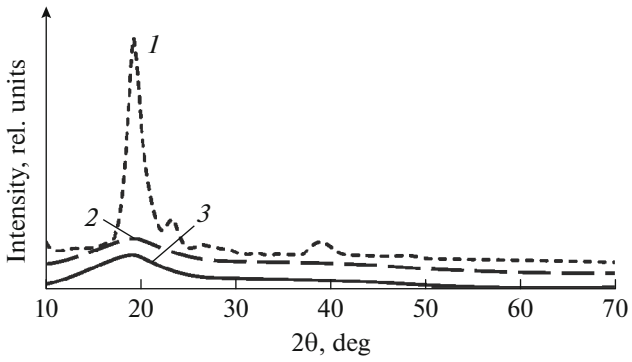

Fig. 4. X-ray powder diffraction patterns of the samples of (*1*) crab chitin and the chitin-containing complexes (*2*) CCC_{SCFE} and (*3*) CCC_{ABH}.

The structures of CCCs were evaluated in comparison with that of crab chitin by X-ray powder diffraction analysis. The X-ray powder diffraction patterns of the samples (Figs. 4) show that crab chitin has a significantly higher degree of crystallinity. The X-ray powder diffraction patterns of the CCCs isolated from the lichen *P. aphthosa* lack pronounced reflections, and in the range $2\theta = 19°–21°$, where the main reflections of crystalline chitin are usually observed, there is a diffuse amorphous halo. The supramolecular structure of CCCs is more defective than that of chitin of animal origin; it is mesomorphic and dominated by amorphous regions, which is consistent with the results of the scanning electron microscopy studies.

The structures of CCCs in comparison with that of crab chitin were also investigated by IR spectroscopy (Fig. 5). The IR spectrum of crab chitin (curve *1*) shows characteristic absorption bands at 3435–3444 cm^{-1} (stretching vibrations of OH groups), 3260–3270 cm^{-1} (stretching vibrations of NH_2 groups), 2800–2920 and 1305–1400 cm^{-1} (vibrations of the C–H bond), 1655 cm^{-1} (stretching vibrations of the C=O bond in the amide group: the Amide I band) and 1560 cm^{-1} (bending vibrations of the C–N bond in the amide group: the Amide II band), and 1000–1050 cm^{-1} (vibrations of the C–O–C bond). The spectroscopic data additionally show the similarity of the chemical structures of the chitinous parts of the studied samples.

Together with the main characteristic bands, the IR spectra of the CCC samples (Fig. 5, curves *1*, *2*) show weak absorption at 890–880 and 800–790 cm, which corresponds to the bending vibrations of the C–H bond in β-sugars and may suggest the presence of glucans in the CCC samples. Moreover, the IR spectra of both isolated CCCs contain a broad band at 3380 cm^{-1}, which is characteristic of melanin and is due to the stretching vibrations of OH groups [49, 50]. At the same time, the peaks in the spectrum of CCC_{ABH} are much weaker than that in the spectrum of CCC_{SCFE} because the acid–base treatment removes a significant part of the proteins and pigments (Table 3).

Potential adsorption sites of CCCs can be primary amino groups ($-NH_2$) and acetamide groups ($-NHC(O)CH_3$) of chitin, carboxyl groups (–COOH) and hydroxyl groups (–OH) of melanin, and glucopyranose cycles of chitin and glucan.

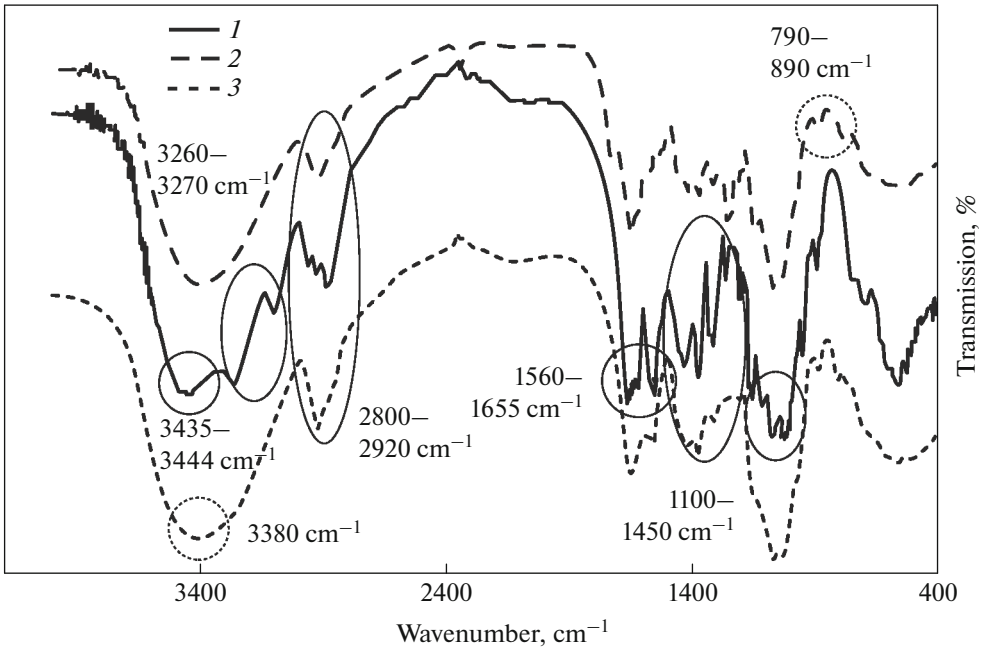

Fig. 5. IR spectra of (*1*) crab chitin and the chitin-containing complexes (*2*) CCC_{SCFE} and (*3*) CCC_{ABH}.

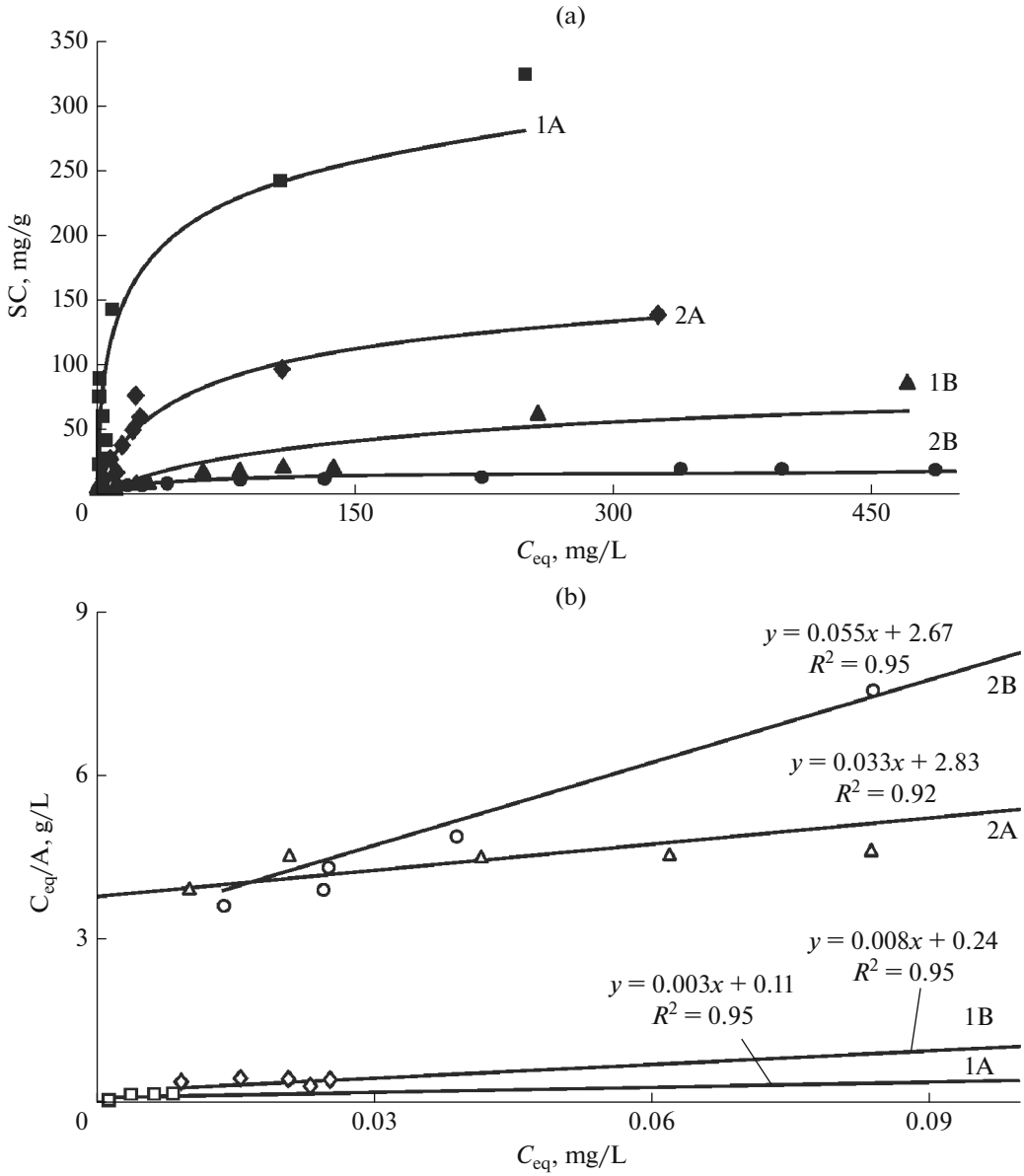

Fig. 6. (a) Adsorption isotherms of the dyes of CCCs from aqueous solutions and (b) their linear anamorphoses in the coordinates of the Langmuir equation: (A) MB, (B) CR, (1) CCC_{ABH}, (2) CCC_{SCFE}.

Table 4. Parameters of the adsorption of dyes on chitin-containing complexes and lichen thallus

Sample	Dye	Adsorption parameter	
		A_∞, mg/g	$K_L \times 10^3$, L/mg
Lichen thallus	MB	90	36
CCC_{ABH}		322	28
CCC_{SCFE}		125	34
Lichen thallus	CR	10	28
CCC_{ABH}		30	11
CCC_{SCFE}		18	19

The sorption properties of the obtained CCCs and the initial lichen raw materials were evaluated using model organic sorbates: the water-soluble dyes MB and CR. These dyes were chosen as model sorbates owing to their wide use for evaluating the adsorption properties of porous materials [51, 52]. Determination of the laws of MB and CR adsorption on the CCC surface makes it possible to predict the processes of adsorption of endotoxins and organic compounds of medium (10–20 kDa) and low (up to 900 Da) molecular weights. MB, being a cationic sorbate, exhibits affinity mainly for materials of an acidic nature, whereas CR, as an anionic sorbate containing a sulfo

group, exists as an anion in aqueous solution and exhibits affinity for materials with basic groups.

Figure 6a presents the adsorption isotherms of MB and CR by the obtained CCCs, which, in accordance with the classification of S. Brunauer, L. Deming, W. Deming, and E. Teller, can be assigned to type I and described by the Langmuir equation [53]. Table 4 lists the adsorption parameters—limiting adsorption A_∞ (mg/g) and sorption equilibrium constant K_L (L/mg)—calculated from the linearized form of the Langmuir equation (Fig. 6b presents the linear anamorphoses of the isotherms).

The initial lichen thallus has a low sorption capacity for both MB and CR: the limiting adsorptions are 90 and 10 mg/g, respectively. The removal of low-molecular-weight extractives during SCFE favors an increase in SC. The limiting capacity reaches 125 mg/g for MB and 18 mg/g for CR. Simultaneously, there is a decrease in the adsorption equilibrium constants K_L, which can characterize the interaction energy of the adsorbate with the adsorbent, provided that the entropy term is constant, i.e., that the similarity of the structures of the adsorption complexes in the considered series of systems. CCC_{ABH} has the highest SC (322 mg/g for MB and 30 mg/g for CR), which is probably related to a deeper development of the structure of the sorbent surface and correlates with the data of the scanning electron microscopy studies (Fig. 3). The obtained CCCs have an affinity for both basic (MB) and acidic (CR) dyes, which indicates their polyampholytic nature.

By and large, the CCC obtained by ABH has a higher sorption capacity compared to the CCC obtained by SCFE (322 vs. 125 mg/g). However, due to the significantly higher yield of the sorbent obtained by SCFE (96 vs. 24.9%), it can be concluded that the lichen thallus biomass is more fully utilized by processing by the SCFE method. The sorbent obtained from one gram of raw material (thallus) by the ABH method can extract up to 80 mg of MB, and in the case of the SCFE method, up to 120 mg of MB (50% more). Thus, the efficiency of raw material processing by SCFE is 50% higher than in comparison with ABH. Moreover, the production of the sorbent by ABH requires significant consumption of acids and alkalis, which leads to the formation of a large amount of wastewater. The SCFE method is devoid of this shortcoming.

CONCLUSIONS

Chitin-containing complexes (CCCs) were obtained from the thallus of the lichen *P. aphthosa* by supercritical fluid extraction (SCFE) and acid–base hydrolysis (ABH). The yields of the complexes under optimal conditions are 96 and 25%, respectively. Analysis of the component compositions of the obtained CCCs demonstrated a high degree of degradation of the thalli in the course of ABH (removal of 23% chitin, 65% melanin, 98.5% protein, and 58.5% minerals), in contrast to the SCFE treatment, which removes exclusively extractives (over 97.5%).

Scanning electron microscopy and X-ray powder diffraction analysis determined that the morphologies of CCC_{ABH} and CCC_{SCFE} are similar, and that their structures are more amorphous compared to crab chitin. IR spectroscopy determined the presence of the main functional groups of chitin, melanin, and glucan in the composition of the obtained CSCs.

The initial lichen thallus has a low sorption capacity. The limiting sorption capacity of CCC_{ABH} (CCC_{SCFE}) reaches 322 (125) and 30 (18) mg/g with respect to MB and CR, respectively. The obtained CCCs have a polyampholytic nature.

The efficiency of processing lichen raw materials to obtain CCCs by the SCFE method is 50% higher than that by the ABH method per in terms of the amount of absorbed sorbate per unit mass of the raw material, taking into account the yield of the sorbent and its sorption capacity.

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REFERENCES

1. V. S. Balabaev, I. A. Glotova, and V. N. Izmajlov, Sovrem. Probl. Nauki Obrazov., Nos. 1-1, 235 (2015).
2. M. D. Mukatova, N. A. Kirichko, and E. N. Romanenkova, Vestn. MGTU **18**, 641 (2015).
3. L. R. R. Berger, T. C. M. Stamford, T. M. Stamford-Arnaud, S. R. C. de Alcántara, A. C. Silva, A. M. Silva, A. E. Do Nascimento, and G. M. de Campos-Takaki, Int. J. Mol. Sci. **15**, 9082 (2014).
4. L. F. Gorovoi and V. N. Kosyakov, Biopolim. Kletka **12** (4), 49 (1996).
5. V. N. Unrod and T. V. Solodovnik, Biopolim. Kletka **17** (6), 526 (2001).
6. *Chitosan,* Ed. by K. G. Skryabin, S. N. Mikhailov, and V. P. Varlamov (Bioinzheneriya Tsentr RAN, Moscow, 2013) [in Russian].

7. R. Honegger and S. Bartnicki-Garcia, Mycol. Res. **95**, 905 (1991).
8. D. E. Gimel'brant, I. S. Stepanchikova, and E. S. Kuznetsova, Nov. Sistem. Nizsh. Rasten. **43**, 150 (2009).
9. M. Kaya, M. Gokhan Hallcl, F. Duman, S. Erdogan, and T. Barane, Nat. Product Res. **29**, 1280 (2015).
10. E. Palmquist, New Phytol. **148**, 11 (2000).
11. *Lichens—Indicators of Radioactive Contamination,* Ed. by L. G. Byazrov (KMK, Moscow, 2005) [in Russian].
12. V. P. Ivshin, T. N. Ivshina, F. F. Sharnina, and S. D. Artamonova, Polymer Sci., Ser. B **49**, 305 (2007).
13. N. A. Ushakova, A. E. Doncov, N. L. Sakina, I. A. Ratnikova, N. N. Gavrilova, N. Yu. Garmash, A. I. Bastrakov, and A. A. Kozlova, Izv. Akad. Nauk, Ser. B, No. 1, 55 (2018).
14. N. R. Meichik and D. V. Vorob'ev, Appl. Biochem. Microbiol. **48**, 307 (2012).
15. D. V. Zhil'tsov, O. S. Brovko, I. A. Palamarchuk, T. A. Boitsova, K. G. Bogolitsyn, and D. G. Chukhchin, Izv. Ufim. Nauch. Tsentra RA, No. 3-3, 9 (2018).
16. A. V. Kazakov, K. M. Zlotnikov, N. G. Vinokurova, and A. K. Zlotnikov, Appl. Biochem. Microbiol. **43**, 449 (2007).
17. *Life of Plants,* Vol. 1: *Introduction. Bacteria and Actinomycetes,* Ed. by N. A. Krasil'nikov and A. A. Uranov (Prosveshchenie, Moscow, 1974) [in Russian].
18. I. I. Osovskaya, D. L. Budilina, E. B. Tarabukina, and L. A. Nud'ga, *Chitin-Glucan Complexes (Physico-Chemical Properties and Molecular Characteristics), The School-Book* (SPbGTURP, St. Petersburg, 2010) [in Russian].
19. O. S. Brovko, I. A. Palamarchuk, T. A. Boitsova, K. G. Bogolitsyn, N. A. Val'chuk, and A. D. Ivakhnov, Fundam. Issled., No. 11, 659 (2015).
20. D. V. Zhil'tsov, O. S. Brovko, K. G. Bogolitsyn, A. D. Ivakhnov, I. A. Palamarchuk, and T. A. Boitsova, Usp. Sovrem. Estestvozn., No. 11-2, 210 (2018).
21. O. S. Brovko, A. D. Ivakhnov, I. A. Palamarchuk, and T. A. Boitsova, Russ. J. Phys. Chem. B **11**, 1306 (2017).
22. T. A. Boitsova, O. S. Brovko, A. D. Ivakhnov, and D. V. Zhiltsov, Russ. J. Phys. Chem. B **14**, 1135 (2020).
23. A. D. Ivakhnov, K. S. Sadkova, A. S. Sobashnikova, and T. E. Skrebets, Russ. J. Phys. Chem. B **13**, 1135 (2019).
24. O. S. Brovko, A. D. Ivakhnov, T. A. Boitsova, and D. V. Zhiltsov, Russ. J. Phys. Chem. B **15**, 1273 (2021).
25. E. B. Kershengol'ts, A. A. Shein, and B. M. Kershengol'ts, Nauka Obrazov., No. 2 (38), 74 (2005).
26. L. S. Kassama, J. Shi, and G. S. Mittal, Sep. Purif. Technol. **60**, 278 (2008).
27. E. Amyrgialaki, D. P. Makris, A. Mauromoustakos, and P. Kefalas, Ind. Crops Products **59**, 216 (2014).
28. M. Derrien, M. Aghabararnejadd, A. Gosselin, Y. Desjardins, P. Angersac, and Y. Boumghar, LWT – Food Sci. Technol. **93**, 79 (2018).
29. K. A. Hviyuzova, N. I. Bogdanovich, N. L. Voropaeva, and V. V. Karpachev, Khim. Rastit. Syr'ya, No. 1, 337 (2020).
30. N. L. Johnson and F. C. Leone, *Statistics and Experimental Design in Engineering and the Physical Sciences, Wiley Series in Probability and Mathematical Statistics* (Wiley, New York, 1977).
31. N. G. Nazarov, *A Practical Guide to Solving Measurement Problems Based on Optimal Measurement Plans* (MGTU im. N. H. Baumana, Moscow, 2007) [in Russian].
32. J. M. Kokosa, A. Przyiazny, and M. Jeanott, *Solvent Microextraction: Theory and Practice* (New York, 2009).
33. *Practical Works on Biochemistry,* Ed. by S. E. Severenin and G. A. Solov'eva (MGU, Moscow, 1989) [in Russian].
34. *Planning an Experiment in Examples and Calculations,* Ed. by N. I. Bogdanovich, L. N. Kuznetsova, S. I. Tret'yakov, and V. I. Zhabin (SAFU, Arkhangel'sk, 2010) [in Russian].
35. T. N. Ivshina, V. P. Ivshin, F. F. Sharnina, and S. D. Artamonova, Appl. Biochem. Microbiol. **45**, 313 (2009).
36. N. V. Sushinskaya and V. P. Kurchenko, Teor. Prikl. Ekol., No. 4, 83 (2019).
37. GOST (State Standard) 23042-2015: Meat and meat products. Methods for determining fat (2019).
38. O. V. Pavlova, E. A. Belova, and T. P. Trotskaya, Nauch. Raboty **2** (46), 121 (2014).
39. *State Pharmacopoeia of the Russian Federation* (Nauch. Tsentr Ekspertizy Sredstv Med. Primen., Moscow, 2008) [in Russian].
40. A. M. Kostina, V. G. Babickaya, and A. G. Lobanok, Prikl. Biokhim. Mikrobiol. **16**, 586 (1978).
41. O. S. Brovko, D. V. Zhiltsov, A. D. Ivakhnov, and M. V. Bogdanov, Russ. J. Bioorg. Chem. **47**, 1424 (2021).
42. A. P. Karmanov, L. S. Kocheva, and M. F. Borisenkov, Butler. Soobshch. **45** (1), 76 (2016).
43. A. V. Lishai, T. A. Savitskaya, N. G. Tsygankova, D. D. Grinshpan, and D. Chen, Zh. BGU, Khim., No. 1, 58 (2021).
44. GOST (State Standard) 4453-74: Activated brightening woody powdery charcoal (1976).
45. V. G. Nikolaev, I. I. Gerashchenko, N. T. Kartel', N. M. Gurina, O. N. Bakalinskaya, V. V. Sarnatskaya, E. A. Snezhkova, K. I. Bardakhivskaya, and L. A. Sakhno, Poverkhnost' **3** (18), 310 (2011).
46. T. D. Khokhlova and L. T. Khien, Mosc. Univ. Chem. Bull. **62**, 128 (2007).
47. V. I. Reshetnikova, Khim.-Farm. Zh. **37** (5), 28 (2003).
48. E. V. Veprikova, S. A. Kuznetsova, G. P. Skvortsova, and M. L. Shchipko, Zh. SFU, Khim. **3**, 286 (2008).
49. E. Harki, T. Talou, and R. Dargent, Food Chem. **58**, 69 (1997).
50. A. Mbonyiryivuze, B. Mwakikunga, S. M. Dhlamini, and M. Maaza, Mater., Chem. Phys. **3** (2), 25 (2015).
51. A. S. Alzaydien, Am. J. Environ. Sci. **5**, 197 (2009).
52. O. Yu. Mukhina, I. A. Piskunova, and A. A. Lysenko, Russ. J. Appl. Chem. **76**, 896 (2003).
53. S. Gregg and K. Sing, *Adsorption, Surface Area and Porosity* (Academic, London, 1982).

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