Sorption Capacity of Chitin and Chitosan-Glucan Biopolymers

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Abstract The goal of this study is to examine sorption capacity of chitin-glucan (ChGC) and chitosan-glucan (CsGC) biopolymer complexes extracted from the fungus *Aspergillus niger* mycelium which is a by-product of citric acid production. It was established that the sorption capacity of diacetylated CsGC with primary amine groups is higher against Pb^{2+} and Cu^{2+} (130-140 mg/g) as compared with ChGC (50-80 mg/g). Three abstract models of adsorption isotherms with a high correlation coefficient correspond to Langmuir (R^2=0.99), Freundlich (R^2=0.96) Models and Micropore Volume Filling Theory (MVF) (R^2=0.95). The Langmuir Model (0.78-0.80 g-mole/kg) makes possible to establish a value of the maximum adsorption capacity more precisely. Higher values of Pb^{2+} and Cu^{2+} adsorption effective capacity by the Dubinin-Radushkevich equation (2.6-5.5 kJ/g-mole) indicate there is an interaction between sorbate and sorbent functional groups forming stable complex chelate structures. The study results show that production of chitin- and chitosan-glucan biopolymers from fungus Aspergillus niger mycelial by-products and their use to remove lead, copper and other heavy metals capable to form chelate compounds from aqueous solutions have highly promising future.

Keywords Sorption of heavy metals, mycelial biomass, chitin, chitosan, sorption isotherms

Introduction

Chitin (poly-N-acetyl-1,4-D-glucosamine) is a cellulose structural analogue and belongs to the most common natural nitrogen-containing polyaminosaccharides. It is the main shellfish component (crabs, shrimps, krill) and form part of the composition of fungal cell wall [1, 2]. Chitin and its diacetylated derivative chitosan with their unique properties attract specific attention nowadays. They can be widely used in biotechnology, medicine, food industry, agriculture and cosmetology [1, 3]. Having a high biological activity they possess wound-healing, antitumour and radioprotective properties. They are able to bind and remove lipids, cholesterol, different toxins and bacteria from the body and can be used as enterosorbents. Besides of that they contain highly active functional amine groups and can form chelate compounds with heavy metal cations, radionuclides and adsorb them [4, 5]. The most important is that these natural biopolymers are biodegradable and safe for human beings and environment.

Chitin is obtained mainly by crustacean shell processing today. Large tonnage mycelial by-products of citric acid production may become an alternative resource of chitin and its derivatives [6]. It is known, the fungus *Aspergillus niger* cell wall contains up to 40% of chitin in the form of chitin-glucan complex [7].

According to the existing data, chitin-glucan biopolymers from fungal biomass possess better sorption capacity and other valuable properties as compared with crustacean chitin [8, 9]. Studies on sorption capacity of chitin- and chitosan-glucan compounds extracted from *Aspergillus niger* fungal mycelial biomass are very important from scientific and practical point of view.

The goal of this paper is to examine sorption capacity and establish an abstract model for adsorption of Cu^{2+} and Pb^{2+} ions by chitin- and chitosan-glucan biopolymers extracted from the mycelial by-product of the citric acid production.

Materials and Methods

Samples of chitin-glucan (ChGC) complex and its diacetylated chitosan-glucan complex (CsGC) were used as study objects. The samples were produced from mycelial by-products of the citric acid biotechnological production by the technology developed in the Institute [8]. ChGC was extracted by biomass acid-alkali treatment, deproteinisation and demineralisation with further removal of hydrolysis products, including associated proteins, lipids, pigments and mineral substances. CsGC synthesis was carried out at ChGC deacetylation by sodium hydroxide concentrated solutions. Deacitylation level was 82% to 95%.

The obtained preparations and their phase composition were identified by X-ray diffraction analysis. A diffractometer SHIMADZU XRD 7000 with the Roentgen tube having copper anode (λ=1.54 Å) was used to make the above analysis. X-ray diffraction patterns were processed with the PDWin software package. The 2θ sliding angle measurement error was not larger than 3'. The 2θ scanning range was 5°–80°. Besides of the above samples, biopolymer complex samples after copper ions Cu^{2+} sorption from $CuSO_4$ solution were scanned as well.

Sorption capacity of ChGc and CsGC samples was evaluated by cuprum (Cu^{2+}) and lead (Pb^{2+}) adsorption from sulfurous and nitrate solutions of these salts correspondingly under static conditions and the temperature regime 20 $\pm$ 2 ℃. The selected samples were intensively powdered before the trial started. Correlation between the solution volume (dm^3) and the sample mass (g) was 1:2. The quantity of adsorbed metal ions was calculated by the difference of metal concentration in the solution before and after sorption. Iodometric titration was used to determine Cu^{2+} concentration in solutions, and calorimetric sulfide method based on optical density measurement in the double-beam spectrophotometer SHIMADZU UV-1800 with 190-1100 nm range was used to determine Pb^{2+} content. The measurement error was 0.1-0.5 mg/dm^3. The observed data statistics was carried out using *Statistica* application package.

Results and Discussion

Light samples were obtained after mycelial biomass hydrolysis at 1:10 liquor ratio and cleaning with a hot water up to neutral filtrate reaction. Melanin (pigments), water-soluble saccharides and fats were removed from the mycelium during hydrolysis. After drying the product obtained a fibrous structure.

The analysis showed that the effective removal of the above impurities during alkaline hydrolysis greatly depends on reagent concentration. Herewith temperature increase and hydrolysis time do not have effect on this process.

As a result ChGC and chitosan were extracted.

When concentrated alkali solutions 20-24% attack the biomass at the subsequent treatment stage, acetamido groups of chitin components are diacetylated and chitosan-glucan complex forms. Subsequent treatment of the obtained complex by acetic acid solution and then by alkali liquor made possible to extract a low-molecular chitosan with a molecular mass of 600 - 20,000 Da. The dried specimens were different by colour: from dark to light beige.

X-ray diffraction patterns were interpreted and the obtained biopolymers were identified. For the chitin-glucan complex the following reflexes are indicative at glazing angles 2Θ: 14.6418; 15.0415; 18.4829; 19.2187; 24.1423; 31.1985; 45.4772. For chitosan 2Θ: 8.6527; 22.8143; 27.0262; 29.1483; 35.7064; 37.5223; 42.8688; 43.7868; 47.2625; 48.2537; 52.5686; 57.1173; 60.3831; 61.1262; 64.2330; 65.3838; 70.0014; 72.6115; 77.3152.

The analysis of the obtained data shows the extracted chitosan contains ChGC fragments (reflexes at $2\Theta = 14.6967^{o}$; 24.1551^{o}; 29.1483^{o}; 30.6670^{o}; 31.2132^{o}; 35.7064^{o}; 37.5223^{o}; 42.8688^{o}; 52.5686^{o}; 60.3831^{o}). So, complete deacitylation was not obtained. Appearance of intensive reflexes at $2\Theta = 29.1483^{o}$; 35.7064^{o}; 39.1349^{o} and 47.2625^{o}; 64.2330 shows there is CsGC together with chitosan.

Sorption isotherms obtained by experiments (Fig. 1-5) correlate the equilibrium sorption value (a, mg/g) and sorbate concentration (c, g/dm^3). Sorption isotherm analysis shows the CsGC sorption capacity is higher than ChGC by Cu^{2+}

(Fig.1) and Pb^{2+} ions (Fig.2) because of primary amine groups (-NH_2) in the chitosan-glucan complex. According to the experimental data amine nitrogen mass fraction in CsGC is 1.5 times higher than in ChGC. Acetylic groups presented in ChGC probably induce additional steric hindrances while sorbing Pb^{2+} and Cu^{2+} ions. The limited Cu^{2+} sorption value on CsGC (140-148 mg/g) exceeds chitosan sorption capacity obtained from crustaceans and is compared with KU-2-8 cationite sorption capacity (130-135 mg/g) [9].
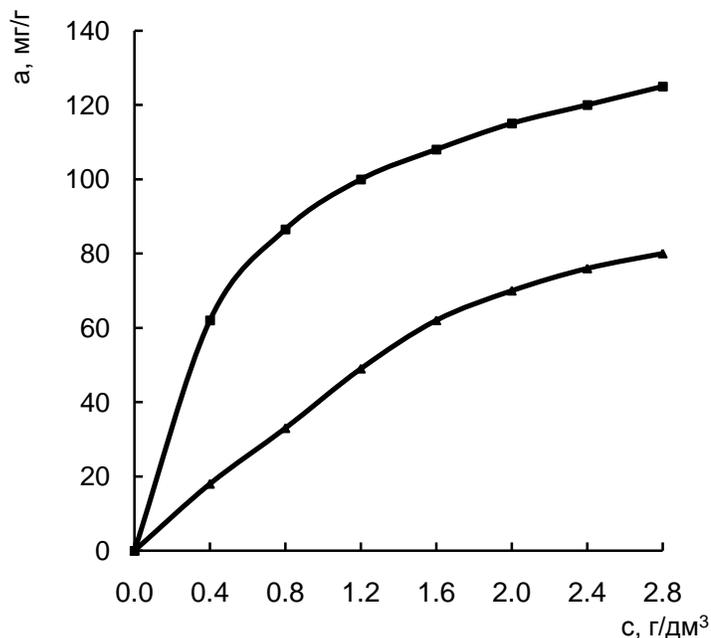

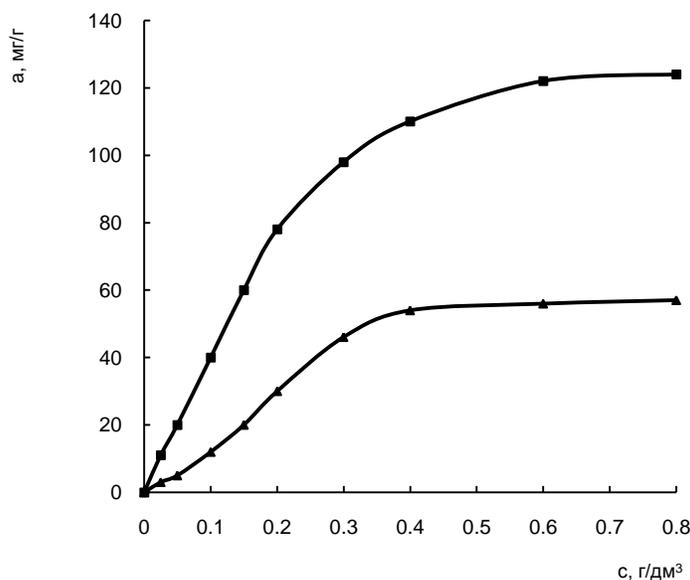*Figure 1: Cu^{2+} ion sorption isotherms ▲ - ChGC; ■ - CsGC*

Figure 2: Pb^{2+} ion sorption isotherms ♦ - ChGC; ▪ - CsGC

Curve characteristics show it is possible to assign the obtained isotherms to the Type I adsorption isotherms in conformity with BDDT classification (Brunauer, Deming, Deming-Teller) [10]. With the aim to revel the trends of Cu^{2+} and Pb^{2+} ions sorption on ChGC and CsGC, experimental isotherms were expressed in mathematical terms using three theoretical adsorption models: Langmuir, Freundlich, Dubinin-Radushkevich, the most frequently

applied to describe equilibrium adsorption processes [10, 11]. Langmuir theory outlines monomolecular adsorption on the solid uniform surface where all adsorption centers are equal (equations 1, 2):

$$a = \frac{a_{max} k_1}{1 + k_1 c},$$ (1)

or in a linear form:

$$\frac{1}{a} = \frac{1}{a_{max}} + \frac{1}{k_1 a_{max} c},$$ (2)

where a_{max} – maximum (limiting) sorption capacity (mg/g), k_1 – Langmuir sorption equilibrium constant, characterising sorption process intensity (dm^3/g-mole).

Langmuir equation, usually with a high correlation coefficient, describes heavy metals equilibrium sorption by polysaccharide sorbents, explaining its frequent use [12].

Freundlich Model is widely used to describe sorption of a dissolved substance from a solution on the solid heterogeneous surface. This process is described by the following empirical equation in the form of power function (3) or as a linear polynomial logarithm (4):

$$a = k_2 c^n,$$ (3)

$$\ln a = \ln k_2 + n \ln c,$$ (4)

where k_2 and n – Freundlich equation constants.

Micropore Volume Filling Theory (MVF) is mainly used to describe adsorption processes on micropore adsorbents [11]. Some authors [12, 13] report the MVF model describes isotherms of Pb^{2+} ions sorption by different polysaccharides. The authors of the paper [14] showed all three models including MVF describe Pb^{2+} ion sorption by *Aspergillus parasiticum* fungal biomass with a correlation coefficient not lower than 0.97. The MVF equation as applied to sorption from solutions (Dubinin-Radushkevich equation) has the following form (5):

$$\ln a = \ln a_{max} - \left[\frac{RT}{E} \ln \frac{c_s}{c_p} \right]^m,$$ (5)

where a – equilibrium quantity of the adsorbed substance at the given temperature (g-mole/kg), c_s – sorbate solubility in water (g-mole/kg of solvent agent), c_p – equilibrium sorbate concentration (g-mole/kg); R – universal gas constant (8,31 J/mole °K), T – Kelvin temperature (°K), E – characteristic adsorption energy (J/g-mole), m – parametre related with adsorbent structure.

Mathematical treatment of the experimental data allowed obtaining sorption isotherms in a linear form graphically represented in coordinates $1/a = f(1/c)$, $\ln a = f(\ln c)$ и $\ln a = (\ln c_s/c)$ (Fig.3-5).
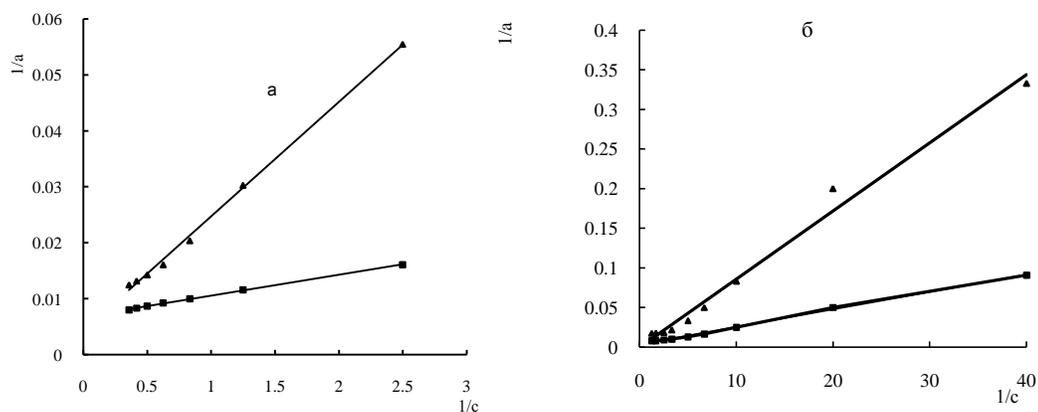

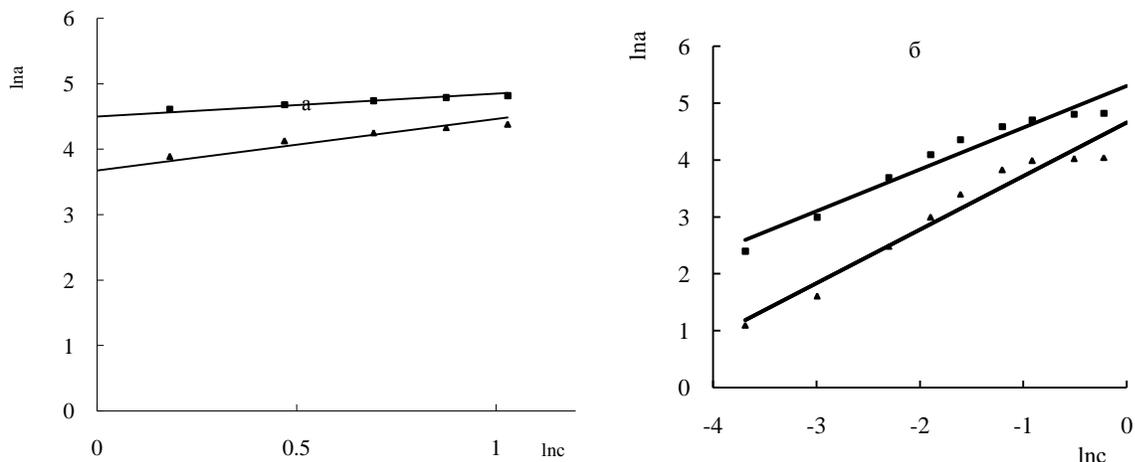*Figure 3: Cu^{2+} (a) and Pb^{2+} (b) ion sorption isotherms in Langmuir equation coordinates: ▲ - ChGC; ■ - CsGC*

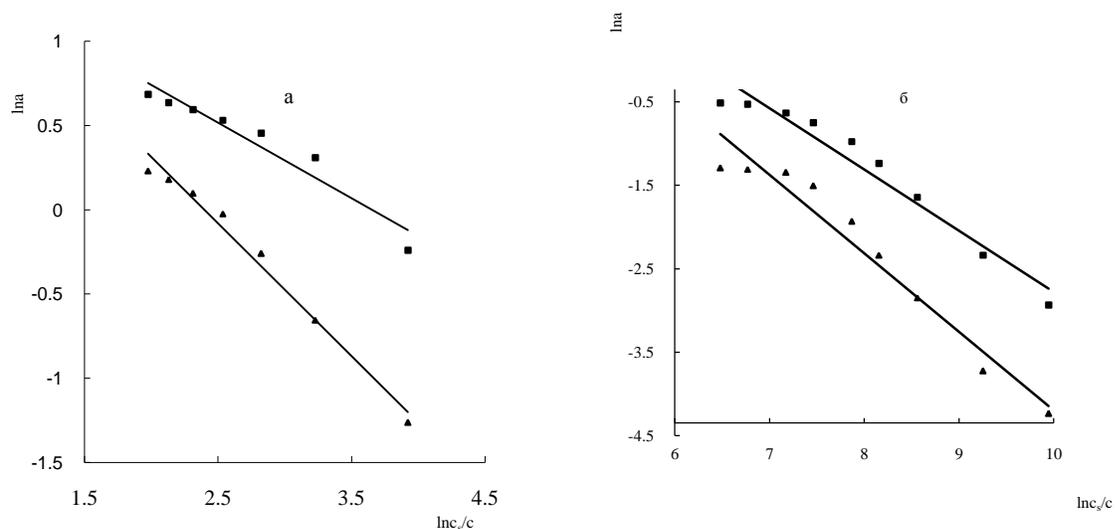*Figure: 4 – Cu^{2+} (a) and Pb^{2+} (b) ion sorption isotherms in Freundlich equation coordinates:* ▲ *- ChGC;* ■ *- CsGC*

Figure 5: Cu^{2+} (a) and Pb^{2+} (b) ion sorption isotherms in Dublin-Radushkevich equation coordinates: ▲ *- ChGC;* ■ *- CsGC*

All three used models well enough describe sorption processes of Cu^{2+} and Pb^{2+} cations by chitin- and chitosan-glucan biopolymers extracted from *Aspergillus niger* fungal mycelia. However Langmuir equation approximates sorption processes under consideration more precisely with a high correlation coefficient (R^2=0.998). Sorption isotherms of Cu^{2+} and Pb^{2+} on ChGC and CsGC are described by Freundlich equation (R^2=0.97 and R^2=0.96 correspondingly) and Dubinin-Radushkevich one (R^2=0.95 and R^2=0.94) with some deviation from the theory. The obtained linear dependences allowed to obtain parametres of Cu^{2+} and Pb^{2+} ion equilibrium sorption on ChGC and CsGC (Tables 1, 2).

Table 1: Parametres of Cu^{2+} ion equilibrium sorption on ChGC and CsGC

Sorbent	Langmuir Model		Freundlich Model		Dubinin-Radushkevich Model	
	a_{max}, g-mole/kg	k_1, dm^3/g-mole	k_2	n	a_{max}, g- mole /kg	E, kJ/g- mole
ХГК	1.27±0.20	38.40±4.3	39.4±4.5	0.79±0.03	66	3.05±0.20
ХТЗ ГК	2.20±0.28	122.85±6.7	89.9±6.3	0.35±0.02	5.14	5.43±0.28

Table 2: Parametres of Pb^{2+} ion equilibrium sorption on ChGC and CsGC

Sorbent	Langmuir Model		Freundlich Model		Dubinin-Radushkevich Model	
	$a_{max,}$ g-mole/kg	$k_{1,}$ dm^3/g-mole	k_2	n	$a_{max,}$ g- mole /kg	E, kJ/g- mole
ХГК	0.38±0.08	305.0±10.6	105.4±8.3	0.94±0.04	95.0	2.59±0.12
ХТЗ ГК	0.71±0.10	640.2±14.4	200.3±10.6	0.73±0.03	183.1	3.32±0.16

Values of Cu^{2+} и Pb^{2+} maximum sorption capacity fixed by Langmuir equation were (80-81) mg/g for ChGC and (140-152) mg/g for CsGC. It should be noted the Dubinin-Radushkevich equation (MVF Theory) could be used to describe processes of Cu^{2+} and Pb^{2+} ion equilibrium sorption on chitin- and chitosan-glucan biopolymer sorbents with a high probability. However if maximum sorption capacity estimated by Langmuir and MVF (by Dubinin-Radushkevich equation where m=1) Models is compared it will be seen Cu^{2+} и Pb^{2+} ion maximum sorption values are something different. It can be explained there is a difference in the regularities of sorption process behavior on micropore and polyaminosaccharides sorbents.

Freundlich constant (n) characterise sorption process intensity. According to its analysis, the energy of sorbent-sorbate bond where n < 1 increase as far as the solid surface is filling. It indicates the sorbent sorption centres are unequal and the mechanism of interaction between functional groups and sorbate is different.

Relatively high values of characteristic sorption energy determined by Dubinin-Radushkevich equation (2.6-5.5) demonstrate the Cu^{2+} and Pb^{2+} sorption on the studied biopolymers is based on the formation of stable sorbate structures with functional sorbent groups. It was found that the mechanism of interaction between metal ions with chitosan-glucan sorbent assumes stable complex chelate compounds of adsorbed ions with carboxy and amino groups form based on the ion and coordinate bond. The same mechanism is set forward in this paper [15].

The relevant X-ray diffraction analysis of CsGC showed the samples after Cu^{2+} sorption have a new phase. Intensive reflexes on X-ray pattern in the field of glazing angles 2Θ equal to 12.4997^o; 25.3969^o; 32.9020^o; 34.0131^o; 36.8401^o and 38.2853^o correspond to this phase. Most probably they refer to the produced complexes. Nevertheless the character of metal ion sorption is very complicated. It is evident besides the most simple and stable complexes – copper-glucosamine-hydroxyl – form, with a neighbour acetyl group less stable complexes are produced (typical for chitin and ChGC). These groups are able to falsify a dimensional orientation of hydrogen bonds taking part in the complex structure formation. It is possible the formed surfaces of ChGC and CsGC samples can adsorb the resulting copper hydroxide. This problem should be investigated at full length.

With that it should be remarked that it is possible to consider X-ray analysis data as a validation of the new phase appearance in CsGC samples after copper ion sorption.

The parametres obtained in the frame of classic equilibrium models are important to understand the sorption mechanism and to enhance efficiency of sorption processes taking course during Cu^{2+} and Pb^{2+} sorption on chitin- and chitosan products extracted fro mycelial biomass.

Conclusion

Chitin- and chitosan-glucan biopolymers were extracted from the food citric acid producer *Aspergillus niger* fungal mycelial mass. They were evaluated for Cu^{2+} and Pb^{2+} sorption capacity. It was established that because of highly active primary amino groups in CsGC composition its sorption capacity is higher than ChGC one. Adsorption isotherms were obtained in the frame of three sorption models during experiments and subjected to the mathematical treatment. The equilibrium sorption of these ions on ChGC and CsGC are well described by Langmuir (R^2=0.99), Freundlich (R^2=0.96) and Dubinin-Radushkevich (R^2=0.95) equations with a high correlation coefficient. This is Langmuir Model that allows establishing the maximum sorption capacity the most precisely.

Parametres of Cu^{2+} and Pb^{2+} equilibrium sorption on ChGC and CsGC were defined. It became possible to establish regularities and the mechanism of sorption interaction between adsorbate and adsorption centres of biopolymer complexes based on the formation of stable chelate ones. Biopolymer identification results obtained by X-ray analysis can be considered as the confirmation there is a new phase in CsGC after copper ion sorption from

solutions. The obtained data show that the use of chitin- and chitosan-glucan complexes as sorbents to extract copper, load and other hard metals from aqueous systems has a promising future.

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