

UDC: 577.114.083

OPTIMISATION OF CONDITIONS FOR DEACETYLATION OF CHITIN-CONTAINING RAW MATERIALS

DOI: <https://doi.org/10.15673/fst.v15i3.2152>

Article history

Received 11.03.2021

Reviewed 28.04.2020

Revised 12.06.2021

Approved 31.08.2021

Correspondence:

M. Trusova
E-mail: bru1.92@mail.ru

Cite as Vancouver style citation

Pavlova O, Trusova M. Optimisation of conditions for deacetylation of chitin-containing raw materials. Food science and technology. 2021;15(3):63-70. DOI: <https://doi.org/10.15673/fst.v15i3.2152>

Цитування згідно ДСТУ 8302:2015

Pavlova O., Trusova M. Optimisation of conditions for deacetylation of chitin-containing raw materials // Food science and technology. 2021. Vol. 15, Issue 3. P. 63-70
DOI: <https://doi.org/10.15673/fst.v15i3.2152>

Copyright © 2015 by author and the journal
"Food Science and Technology".

This work is licensed under the Creative Commons
Attribution International License (CC BY).
<http://creativecommons.org/licenses/by/4.0>



Introduction. Formulation of the problem

Producing food-grade citric acid results in tons of waste biomass of the fungus *Aspergillus niger*, which is a valuable renewable secondary raw material resource. The cell walls of *Aspergillus niger* can contain up to 40% of chitin, which is a precursor of chitosan, a valuable natural sorbent [1]. Chitosan can have various applications in the food industry, in particular, as a sorbent and stabiliser in beer manufacture.

Analysis of recent research and publications

Chitin is a homopolymer of β -(1 $\rightarrow$ 4)-linked N-acetyl-D-glucosamine. Its structure is almost identical to that of cellulose, the difference being in the presence

O. Pavlova¹, Candidate of Technical Sciences, Associate Professor
M. Trusova², graduate student of the department of technologies of
alcoholic and non-alcoholic products

¹Department of Technology, Physiology and Food Hygiene
Educational Institution "Grodno State University
named after Yanka Kupala"

3/1, Lane. Dovatora, Grodno, Republic of Belarus, 230029

²Scientific and Practical Food Center of the National Academy of
Sciences of Belarus

29, st. Kozlova, Minsk, Republic of Belarus, 220037

Abstract. The paper describes the differences between chitosan and chitin, and reviews works by foreign scientists on obtaining chitosan from various raw materials. Methods of modifying chitosan and obtaining combined sorbents have been analysed. It has been studied whether chitosan is applicable in the technology of wines and alcoholic beverages as a sorbent. The purpose of the study was determining the optimal conditions of the deacetylation stage to obtain chitosan with the best sorption properties from *Aspergillus niger* biomass. A three-factor experiment has been carried out. It involved obtaining 27 samples of chitosan using sequential four-step acid-base hydrolysis under various conditions of the deacetylation stage. The deacetylation process was optimised under alkaline conditions depending on the alkali concentration, processing temperature, and exposure. For each of the samples obtained, the adsorption activity, specific surface area, and distribution coefficient in the sorbent-sorbate system have been determined. The degrees of deacetylation of all chitosan samples have been determined by potentiometric titration. The study has resulted in determining the optimal conditions for the deacetylation stage: processing temperature 110–130°C, sodium hydroxide concentration 27–36 g/dm³, exposure 45 to 65 minutes. The sample deacetylated at the temperature 120 °C, alkali concentration 30 g/dm³, and exposure 45 minutes has shown the best adsorption activity values: the adsorption activity for methyl orange 347.96 mg/g, the specific surface area of the sorbent samples 0.52·10⁵ m²/g, the distribution coefficient in the sorbent-sorbate system 3.29·10⁻³ ml/g. This sample had the highest degree of deacetylation, 43.6%. The sample has been analysed using IR spectroscopy, and its main characteristic frequencies have been studied. It has been concluded that the sample obtained was equivalent to the reference chitosan.

Keywords: chitosan, deacetylation, methyl orange, adsorption activity, three-factor regression analysis.

of an amino group (-NH₂) instead of hydroxyl (-OH) in the C₂ position. The simplest chitin derivative is chitosan, an amino polysaccharide (2-amino-2-deoxy- β -D-glucan) obtained by deacetylation of chitin [2]. The difference between chitin and chitosan lies in their acetyl group contents. Chitosan has positive ionic charges, which allows it to bind chemically with negatively charged lipids, cholesterol, metal ions, proteins, and macromolecules [3-5]. The chemical structure of chitosan is that of an N-acetyl-D-glucosamine and D-glucosamine copolymer. Chitosan is a high-molecular-weight polymer, non-toxic and biodegradable.

M. Hossain and A. Iqbal described the chemical process of producing chitosan from shrimp waste

through demineralisation, deproteination, and deacetylation. The chitosan quality was proved to depend on the conditions of chemical extraction. The results showed 3% of HCl and 4% of NaOH to be the most suitable concentrations for demineralisation and deproteination respectively. Highly soluble chitosan with a high deacetylation degree was obtained by deacetylation with 60% NaOH solution at 60°C for 24 hours [6]. Abdou *et al.* obtained chitin from six different local sources in Egypt. The resulting chitin was converted into a more useful soluble chitosan by deacetylation in NaOH solutions of various concentrations. The alkaline chitin was then heated in an autoclave, which reduced the deacetylation time dramatically [7]. The authors [8] conducted an experiment to obtain chitosan from insects, particularly those from the orders *Lepidoptera*, *Coleoptera*, *Orthoptera*, *Hymenoptera*, *Diptera*, *Hemiptera*, *Dictyoptera*, and *Odonata*. Besides, the methods of extraction and the physicochemical properties of the sorbents obtained were studied. It was proved that chitin and chitosan obtained from insects had numerous biological activities and nutritional, biomedical, and industrial applications. Y. Tan *et al.* cultured *Mucor circinelloides* on a red grape pomace substrate. The physicochemical properties of the fungal chitosan extracted were determined by IR spectroscopy and elemental analysis. Fungal chitosan extracted by *Mucor circinelloides* fermentation had a deacetylation degree comparable to that of commercial chitosan [9]. Summarising all the above, chitosan can be obtained from the shells of crustaceans (crabs and shrimps) or from the exoskeleton of insects, but the most promising raw material is fungal mycelium [10]. The availability of such a raw material source as *Aspergillus niger* mycelium (citric acid production waste) makes the research into chitosan applications and improvement of chitosan production very promising [11-13].

There are a number of methods to produce chitosan. The classic one is acid-base hydrolysis. It includes three stages (deproteination, demineralisation, and deacetylation), during which acetyl groups are partially removed from the polymer chain [14,15]. The key stage in the chitosan production process is deacetylation. The properties of resulting chitosan can differ depending on the processing conditions, temperature, concentration of the reagents, and time.

The researchers [16] reviewed and compared various methods of producing chitosan and tested some ways of improving the functional properties of chitosan and controlling its physicochemical properties by obtaining various modifications of the sorbent. Preparation of poly aniline modified chitosan and application of a nanocomposite to remove Cu (II) from aqueous solutions were studied by the researchers from the Islamic Azad University [17]. A large-scale study to obtain modified chitosans was carried out by N. A. Negm *et al.* The synthesis resulted in obtaining substituted chitosan derivatives (including thiolated,

phosphorylated, and N-phthaloylated derivatives), cross-linked chitosan derivatives (including chitosan-glutaraldehyde, chitosan-ethylenediaminetetraacetic acid, and chitosan-epichlorohydrin derivatives), carboxylic acid derivatives of chitosan obtained by chitosan carboxyalkylation, acrylation, methacrylation, and benzoylation, ionic chitosan derivatives (including highly cationic and sulphated derivatives), and chitosan bound to specific molecules (cyclodextrin, thiosemicarbazone, dioxime, and crown ether precursors) [18].

Along with studying how to produce chitosan and improving its properties, researchers are focusing on how the sorbent can be applied in various economic activities. The most promising area is the food industry, especially the manufacture of fermented beverages, beer, and distilled beverages. Chitosan as a sorbent can be used for sorption of protein and polyphenolic substances to prevent the cloudiness of the finished beverage. Chitosan contains a lot of amino and hydroxyl groups and is highly reactive, which makes it applicable in this area. Since vodka, beer, distilled beverages, and kvass are colloidal systems, they can develop turbidity of various nature when stored and exposed to various factors [19,20]. This can be due to the presence of proteins and polyphenols in the solution. Negatively charged polyphenols bind to positively charged protein compounds, which leads to turbidity. Chitosan's ability to remove polyphenols and proteins can be explained by the sorbent's chemical structure. Being an active cationic agent, chitosan will effectively remove negatively charged polyphenolic substances from the beverage dispersion. Protein coagulation by chitosan will proceed in two ways. The proteins with the isoelectric points in the pH range 1–5 will be precipitated by direct coagulation, while the proteins with the isoelectric points in the pH range 5–14 will be precipitated via secondary coagulation. First, protein–polyphenol complexes are formed, and then they are precipitated in the presence of a stabiliser [21]. Therefore, the complexing and ion-exchange properties of chitosan can prove helpful for the further advancement of the fermentation industries, including beer manufacture. Some studies indicate that the charge of chitosan also promotes its binding into strong complexes with bacterial and yeast cells, as well as with counterions, including mineral acid anions (phosphates, sulphates, sulphites, etc.) [22].

Based on the above results obtained by domestic and foreign authors, it can be concluded that the key focus of scientific research on chitosan is the search for new sources of it and development of modified chitosans. *Aspergillus niger* mycelium as a source of chitosan has been so far understudied, so the research in this area is important and promising. Also, according to the authors of this study, optimisation of the deacetylation stage will greatly improve the adsorption activity without chitosan modification, which will significantly reduce its prime cost.

The purpose of the study was to determine the optimal deacetylation conditions to produce chitosan with the best sorption properties from *Aspergillus niger* biomass. To achieve the purpose, the following **objectives** were set:

- to obtain chitosan samples from *Aspergillus niger* biomass using sequential four-step acid-base hydrolysis;
- to test the adsorption activity of the samples obtained;
- to determine the values of the adsorption activity and deacetylation degree;
- to analyse the chitosan sample with the best sorption performance.

Research materials and methods

We used commercial samples of inactivated *Aspergillus niger* biomass B-1 obtained by submerged cultivation of citric acid in molasses media at the JSC Skidel Sugar Factory, Skidzel, Grodno Region, Republic of Belarus. At the first stage of the study, chitosan was extracted from *Aspergillus niger* biomass using sequential four-step acid-base hydrolysis. Demineralisation consisted in treating raw biomass with a 3% HCl solution at 30°C for 1 hour. During deproteination, the intermediate product obtained after the first stage was treated with a 2% NaOH solution at 90°C for 1 hour. The third stage consisted in treating the resulting intermediate product with a 6% H₂O₂ solution at 40°C for 1 hour. After each processing stage, the samples were washed with distilled water to achieve the pH 7 and manually squeezed dry through a nylon filter [23]. The final stage was drying the resulting product on cellophane at 20°C and further homogenising it at 20°C for 1 hour (Homogeniser type MPM-302, Poland).

The adsorption activity of the resulting raw material was tested by means of methyl orange (the specification in accordance with GOST 4453-74).

To determine the specific surface area of the sorbent samples (metres per 1 gram of the product), the following formula was used:

$$S_{sp} = A \times S \times N_A$$

where:

S_{sp} is the specific surface area of the sorbent samples (m²/g);

A is the amount of indicator sorbed (mg/g);

S is the area occupied by one sorbent molecule in a monolayer (0.64 · 10⁻¹⁸ m²);

N_A is the Avogadro number (6.02 · 10²³).

The distribution coefficient in the sorbent–sorbate system was determined by the formula:

$$K_d = \frac{CE}{C_{кон.}}$$

where K_d is the distribution coefficient (cm³/g);

CE is the adsorption activity (mg/g);

C_{fin} is the final concentration (mg/dm³).

The deacetylation degree of chitosan was determined by potentiometric titration of hydrogen chloride bound to amino groups of chitosan molecules. Potentiometric titration with 0.1 N solution of sodium hydroxide was performed using an I-160MP ionometer (at 0.1 cm³ increments). The volume of sodium hydroxide used for titration was determined based on the curve showing the dependence between the solution conductivity and the volume of alkali. Before the assay, the ionometer was adjusted against standard buffer solutions. 0.2 g chitosan was transferred into 20 ml 0.1 N hydrogen chloride solution and stirred on a magnetic stirrer for 60 minutes. The resulting solution was then titrated with sodium hydroxide solution till the pH was 11. Analysis of the titration curve has shown that the first break of the curve corresponds to the excess of hydrogen chloride, while the second break corresponds to the amino group concentration in a weighed portion of chitosan. The deacetylation degree was calculated by the formula:

$$DD = \frac{203 \times V \times T}{G - 161 \times V \times T + 203 \times V \times T} \times 100\%$$

Where G is the weighed portion of chitosan, g;

V is the volume of sodium hydroxide solution determined by the difference between the volumes of the second and the first breaks on the potentiometric titration curve, cm³;

161 is the molecular weight of a chitosan elementary unit;

203 is the molecular weight of an acetylated unit of chitosan;

T is the titre of NaOH solution determined by blank titration with 0.1 N hydrogen chloride.

To confirm that the samples obtained are equivalent to the reference chitosan (its spectrum was taken from the IR-Spectrum library), the samples were tested by IR spectroscopy using a Fourier transform spectrometer Thermofisher Scientific Nicolet is50 (ATR FTIR) in the range 400 to 4000 cm⁻¹. Prior to the assay, the sample was compacted into KBr pellets at 650 kgf/cm² for 1 min with a manual press MHP-1. The results were analysed using the Omnic software.

The deacetylation stage of chitosan production was optimised under alkaline conditions, depending on the alkali concentration, temperature, and exposure. When selecting the test factors, it was taken into account whether a factor was controllable (a constant value of a parameter could be set and maintained), compatible (not disrupting the technological process), independent (set independently of other factors), and unambiguous (the factors should not be functions of each other). The behaviour of the factors selected is shown in Table 1.

Table 1 – Variation of the environmental factors

Factor	Code designation of a factor	Level			Variation increment (λ)
		Lowest (-)	Medium (0)	Highest (+)	
Temperature, °C	X ₁	90	120	150	30
Sodium hydroxide concentration, %	X ₂	25	30	35	5
Exposure, min	X ₃	30	45	60	15

The experiments were designed, and the experimental data processed using the software STATISTICA 10.0 and STATGRAPHICS.

Results of the research and their discussion

A full factorial design (FFD) matrix was prepared, according to which 3³ variants of the deacetylation modes were determined. Table 2 shows the three-factor design matrix that allows assessing the effect of the modes on the parameter monitored (adsorption activity, mg/g), and presents the experimental results obtained.

Table 2 – Three-factor design matrix and the results obtained (n = 3, P = 0.1297, R² = 0.9233)

Sample No.	Test factors			Adsorption activity, Y, mg/g
	X ₁ , t	X ₂ , °C	X ₃ , τ	
1	-1	-1	-1	340.23
2	0	-1	-1	343.54
3	1	-1	-1	341.34
4	-1	0	-1	341.89
5	0	0	-1	346.30
6	1	0	-1	340.78
7	-1	1	-1	341.89
8	0	1	-1	345.20
9	1	1	-1	341.34
10	-1	-1	0	340.78
11	0	-1	0	344.65
12	1	-1	0	341.89
13	-1	0	0	342.44
14	0	0	0	347.96
15	1	0	0	341.89
16	-1	1	0	342.99
17	0	1	0	346.30
18	1	1	0	342.99
19	-1	-1	1	342.44
20	0	-1	1	345.20
21	1	-1	1	342.44
22	-1	0	1	342.99
23	0	0	1	346.85
24	1	0	1	342.44
25	-1	1	1	343.54
26	0	1	1	346.85
27	1	1	1	343.54

Regression analysis has shown that R²=0.9233, the standard error of estimation is 0.7637, and the mean absolute error is 0.5081. The statistical significance of each factor has been established by comparing the mean square value with the experimental error estimation. The three test factors have the P-values less than 0.05, which indicates their statistical significance.

Fig. 1 shows the Pareto chart that allows comparing the effect of the test factors on the response function and assessing the significance of the regression equation coefficients obtained. In the chart, the estimates of the effect are arranged according to the absolute values (largest to smallest), and the direction of the factor's effect is also shown.

The Pareto chart presented in Fig. 1 shows that the effect of the test factors on the adsorption activity of the obtained chitosan is not unidirectional: an increase in time and alkali concentration leads to an increase in the adsorption activity of the samples, but the optimal temperature is 120°C. The chart also confirms the significance of the regression equation coefficients.

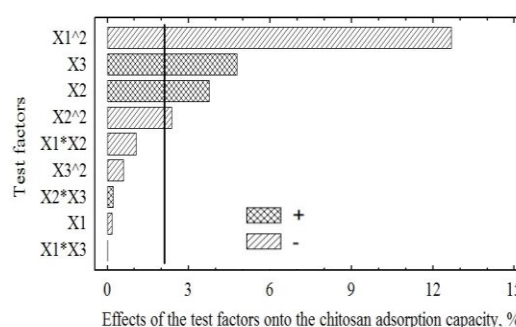


Fig. 1. Standardised Pareto chart for chitosan adsorption activity

The analysis has also allowed calculating the coefficient of determination (R²=0.9514). It confirms the validity of the mathematical model obtained, which can be used to predict the values of the performance indicator. Fig. 2 presents the chart showing the effect of the factors on the adsorption activity.

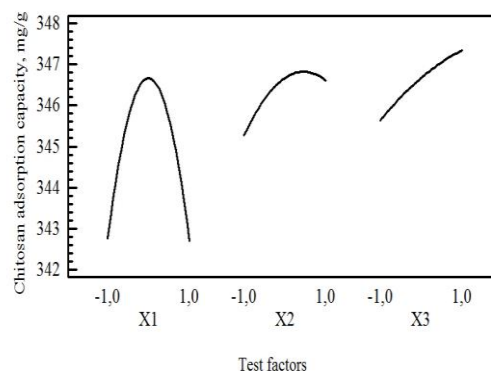


Fig. 2. Effect of the temperature of treatment, NaOH concentration, and exposure on the adsorption activity of chitosan

The chart shows that the greatest effect on the chitosan adsorption activity is that of the sodium hydroxide concentration (positive linear dependence), a lesser one is that of the temperature (positive curvilinear dependence), and the lowest is that of the exposure (U-shaped curvilinear dependence).

The three-factor experiment has shown that the adsorption activity of chitosan is significantly affected by all the three factors (exposure, temperature, and sodium hydroxide concentration). The results obtained can be explained by the following. When the sodium hydroxide concentration exceeded 35%, it resulted in deacetylation of not only the amorphous polymer regions but also of the crystalline ones. This was due to the diffusion of sodium hydroxide closer to the surface of the macromolecule or into it, which directly depended on the alkali concentration. At above 130°C and with prolonged exposure, thermal oxidative destruction takes place. It leads to the loss of low-molecular-weight chitosan fractions with the highest adsorption activity. On the contrary, with the alkali concentration below 25% and insufficient treatment time, polymer's deacetylation degree did not reach high values. Chitosan with the maximum adsorption activity (347.96 mg/g) was obtained at the temperature 120°C, alkali concentration 35%, and exposure 45 minutes. The results are consistent with [24], where chitosan obtained under similar conditions showed a high adsorption capacity for copper ions. The adsorption activity determined on the basis of Cu^{2+} sorption ranged from 190 to 220 mg/g chitosan. The adsorption performance of chitosan is due to its high hydrophilicity and ability to be involved in complex multistage processes of catalysis, dissolution, swelling, surface chemical reactions, and transmembrane transport of low-molecular-weight substances [12].

Based on the experiments, a regression equation has been obtained, which adequately describes the relationship between the adsorption activity of chitosan obtained and the deacetylation modes, and provides an empirical mathematical model of the deacetylation stage:

$$Y = 337.14 - 0.001 \times X_1 + 0.14 \times X_2 + 0.05 \times X_3$$

Where Y is the adsorption activity, mg/g;

X_1 is the temperature, °C;

X_2 is the concentration, g/dm³;

X_3 is the exposure, min.

The model parameters can be interpreted as follows: a 1°C increase in the temperature decreases the adsorption activity by 0.001 mg/g on average; a 1 g/dm³ increase in the concentration increases the adsorption activity by 0.135 mg/g on average; a 1 minute increase in the exposure increases the adsorption activity by 0.051 mg/g on average.

The response surfaces allow selecting the factor variation ranges that ensure achieving the maximum adsorption activity.

The response surface that visualises the adsorption activity as a function of the exposure and temperature is shown in Fig. 3.

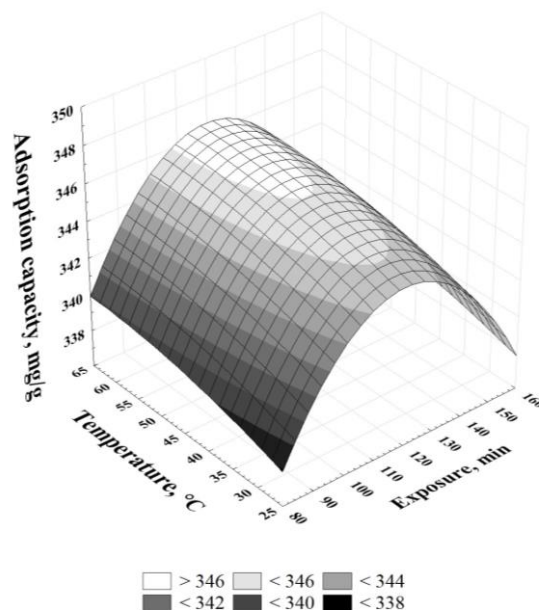


Fig. 3. Response surface of the chitosan adsorption activity as a function of the temperature of treatment and exposure

Analysis of the response surface in Fig. 3 suggests that the maximum chitosan adsorption activity (>346 mg/g) is achieved under the following deacetylation conditions: exposure 45–65 minutes, temperature of treatment 110–130°C.

The response surface that shows the adsorption activity as a function of the exposure and alkali concentration is presented in Fig. 4.

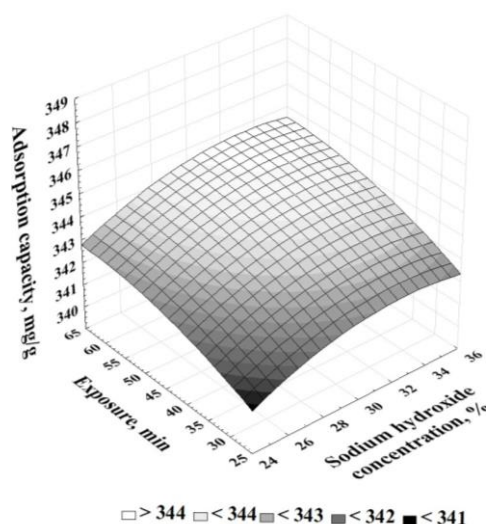


Fig. 4. Response surface of the chitosan adsorption activity as a function of the sodium hydroxide concentration and exposure

As seen from the figure above, the maximum chitosan adsorption activity (>344 mg/g) can be

achieved under the following deacetylation conditions: exposure 45–65 minutes, sodium hydroxide concentration 27–36 g/dm³.

The response surface that shows the adsorption activity as a function of the temperature and alkali concentration is visualised in Fig. 5.

The response surface in Fig. 5 shows the adsorption activity as a function of the temperature and alkali concentration. As is evident from it, these two factors have the greatest effect on the parameters of chitosan adsorption activity. To obtain chitosan with the adsorption activity >346 mg/g, the optimal temperature is 110–130°C and the optimal sodium hydroxide concentration is 27–36 g/dm³.

Such characteristics of the chitosan adsorption activity have been established and analysed as the specific surface area of the sorbent samples, the distribution coefficient in the sorbent–sorbate system, and the deacetylation degree of the chitosan samples. These values are given in Table 3.

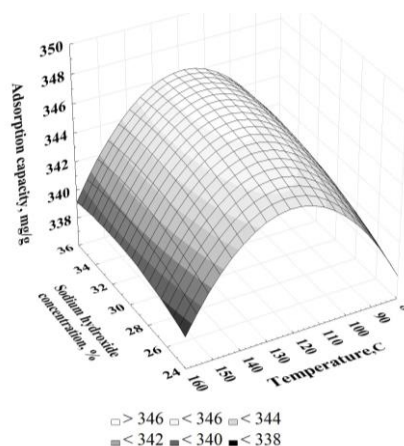


Fig. 5. Response surface of the chitosan adsorption activity as a function of the sodium hydroxide concentration and the temperature of treatment

Table 3 – Adsorption activity parameters and degrees of deacetylation of the chitosan samples

Sample No.	Deacetylation degree, %	Specific surface area of sorbent samples, *10 ⁵ m ² /g	Distribution coefficient in the sorbent–sorbate system, *10 ⁻³ cm ³ /g
1	42.2	0.15	2.49
2	42.6	0.23	2.63
3	42.6	0.21	2.58
4	42.6	0.23	2.63
5	43.2	0.26	2.68
6	42.5	0.21	2.58
7	42.6	0.26	2.68
8	43.1	0.28	2.73
9	42.8	0.28	2.73
10	42.4	0.34	2.84
11	43.2	0.36	2.90
12	42.7	0.41	3.02
13	42.6	0.49	3.22
14	43.6	0.52	3.29
15	42.7	0.36	2.90
16	42.8	0.41	3.02
17	43.2	0.44	3.08
18	42.8	0.18	2.53
19	42.9	0.21	2.58
20	43.1	0.23	2.63
21	42.8	0.15	2.49
22	42.7	0.21	2.58
23	43.3	0.23	2.63
24	42.7	0.18	2.53
25	42.9	0.26	2.68
26	43.2	0.28	2.73
27	42.7	0.13	2.45

The data in the table make it clear that Sample 14 has the highest deacetylation degree (43.6%). The adsorption activity performance directly depends on the deacetylation degree: the higher the deacetylation degree is, the larger the number of free amino groups capable of chemical interaction. These data on the deacetylation degrees correlate with those on the adsorption activity ($P < 0.001$). The values of the specific surface area of the sorbent samples and the

distribution coefficient in the sorbent–sorbate system support the conclusion that the optimal conditions for deacetylation and production of chitosan with high adsorption performance are the temperature 120°C, the sodium hydroxide concentration 30 g/dm³, and the exposure time 45 minutes.

Sample 14 obtained under the optimal deacetylation conditions has been analyzed by IR spectroscopy. The results are shown in Fig. 6.

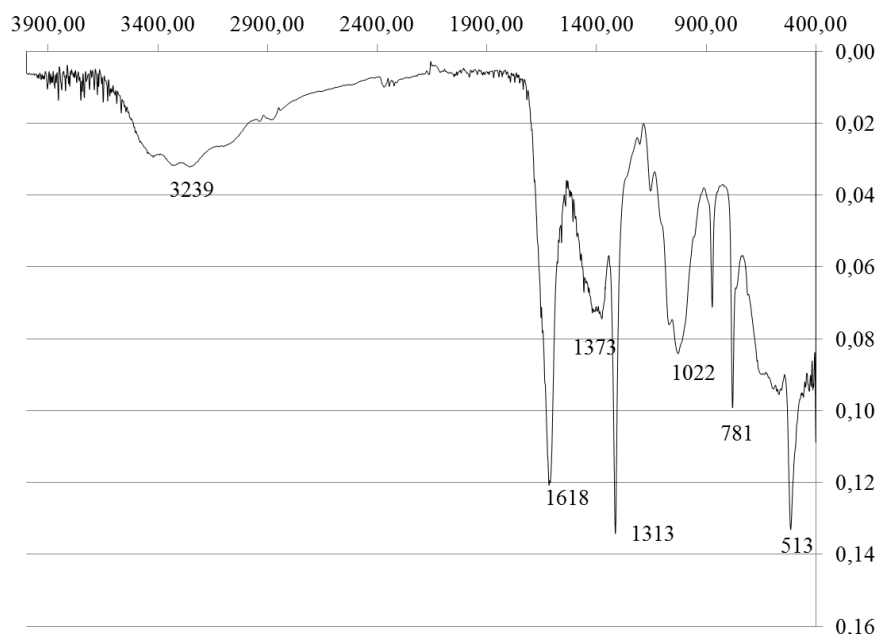


Fig. 6. IR-spectrum of chitosan Sample 14

Based on Fig. 6, let us review the main characteristic frequencies of the sample. The bands at 3.239 and 1.618 cm^{-1} can be seen as amino group vibrations. The region at 3.400 cm^{-1} , too, is overlapped by free hydroxyl vibrations. Vibrations in the range 2050–1618 cm^{-1} correspond to the area of asymmetric NH_3^+ bending. Vibrations at 781–513 cm^{-1} can be interpreted as $\text{C}_1\text{-H}$ bond in β -sugars. Upon comparing the obtained spectrum with the data from literature [25], a conclusion can be made that the quality of the sample obtained is equivalent to that of the reference chitosan. The results of our study are largely comparable with those of numerous adsorption performance studies of chitosan produced from other raw materials [26–32].

Conclusion

During the study, 27 different chitosan samples were obtained from *Aspergillus niger* biomass by means of sequential four-step acid-base hydrolysis.

The adsorption activity of all chitosan samples obtained has been determined. It varied in the range 340.23–347.96 mg/g. The best results are those of Sample 14 which was deacetylated at the temperature 120°C, the alkali concentration 30 g/dm³, and the exposure 45 minutes (the adsorption activity by methyl orange was 347.96 mg/g).

The specific surface area of the 27 sorbent samples ranged from $0.13 \cdot 10^5$ to $0.52 \cdot 10^5$ m²/g, and the distribution coefficient in the sorbent–sorbate system ranged from $2.45 \cdot 10^{-3}$ to $3.29 \cdot 10^{-3}$ cm³/g. The deacetylation degree of the chitosan samples obtained ranged from 42.2 to 43.6%. The best results were observed for Sample 14 deacetylated at the temperature 120°C, the alkali concentration 30 g/dm³, and the exposure 45 minutes: the specific surface of the sorbent samples was $0.52 \cdot 10^5$ m²/g, the distribution coefficient in the sorbent–sorbate system was $3.29 \cdot 10^{-3}$ ml/g, and the deacetylation degree was 43.6%. The data obtained on the deacetylation degree correlate with the adsorption activity values ($P < 0.001$); the higher the deacetylation degree, the better adsorption performance is observed.

Sample 14 has been analysed using IR spectroscopy, and the main characteristic frequencies of the sample have been considered (the bands at 3.239 and 1.618 cm^{-1} correspond to the amino group vibrations, the range at 3.400 cm^{-1} corresponds to the free hydroxyl vibrations, the range at 2.050–1.618 cm^{-1} corresponds to NH_3^+ vibrations, and vibrations at 781–513 cm^{-1} correspond to the $\text{C}_1\text{-H}$ bond in β -sugars). This sample is equivalent to the reference chitosan, the spectrum of which is taken from the IR-Spectrum library.

References:

- Novinyuk LV, Velinzon PZ, Kulev DK. Sorption properties of chitin- and chitosan-glucan bio-complexes isolated from aspergillus niger fungal mycelia biomass. PROCEEDINGS OF UNIVERSITIES APPLIED CHEMISTRY AND BIOTECHNOLOGY. 2017;7(3):64-71. DOI: <https://doi.org/10.21285/2227-2925-2017-7-2-64-71>.
- Gal'braj LS. Hitin i hitozan: stroenie, svojstva, primenenie. Soroskij obrazovatel'nyj zhurnal. 2001;7(7):51-56.
- Gamygin EA, Perednya AA, SHol' AV. Novye kombikorma dlya akva-kul'tury. Zooindustriya [Internet]. 2001 [data obrashcheniya 2021 Sent 15] 2021. Available from: www.vetorg.net/magazines/3/2001/30/84
- Kaur S, Dhillon G. The versatile biopolymer chitosan: potential sources, evaluation of extraction methods and applications. Critical Reviews in Microbiology. 2013;40(2):155-175. DOI:<https://doi.org/10.3109/1040841X.2013.770385>
- Bilbao-Sainz C, Chiou B, Williams T, Wood D, Du W, Sedej I et al. Vitamin D-fortified chitosan films from mushroom waste. Carbohydrate Polymers. 2017;167:97-104. DOI:<https://doi.org/10.1016/j.carbpol.2017.03.010>.

6. Hossain M, Iqbal A. Production and characterization of chitosan from shrimp waste. *Journal of the Bangladesh Agricultural University*. 2021; 12(1):153-160. DOI: <https://doi.org/10.3329/jbau.v12i1.21405>
7. Abdou ES, Nagy KSA, Elsabee MZ. Extraction and characterization of chitin and chitosan from local sources. *Bioresource Technology*. 2008;99(5):1359-1367. DOI: <https://doi.org/10.1016/j.biortech.2007.01.051>
8. Mohan K, Ganesan AR, Muralisankar T, Jayakumar R, Sathishkumar P, Uthayakumar V, et al. Recent insights into the extraction, characterization, and bioactivities of chitin and chitosan from insects. *Trends in Food Science & Technology*. 2020;105:17-42. DOI: <https://doi.org/10.1016/j.tifs.2020.08.016>
9. Tan YN, Lee PP, Chen WN. Dual extraction of crustacean and fungal chitosan from a single mucor circinelloides fermentation. *Fermentation*. 2020;6(2):40. DOI: <https://doi.org/10.3390/fermentation6020040>
10. Hamdi M, Nasri R, Li S, Nasri M. Bioactive composite films with chitosan and carotenoproteins extract from blue crab shells: Biological potential and structural, thermal, and mechanical characterization. *Food Hydrocolloids*. 2019;89:802-812. DOI: <https://doi.org/10.1016/j.foodhyd.2018.11.062>
11. Liao J, Huang H. Extraction of a novel fungal chitin from hericium erinaceus residue using multistep mild procedures. *International Journal of Biological Macromolecules*. 2020;156:1279-1286. DOI: <https://doi.org/10.1016/j.ijbiomac.2019.11.165>
12. Pavlova OV, Gladkaya OK, Trusova MM. Vliyanie usloviy sorbcii na sorbcionnyuyu aktivnost' hitozana. *Pishchevaya Promyshlennost': Nauka i Tekhnologii*. 2019;12(4):86-93.
13. Divya K, Rebello S, Jisha MS. A simple and effective method for extraction of high purity chitosan from shrimp shell waste. *Proceedings of the International Conference on Advances in Energy, Environment and Chemical Engineering*. 2014;1(4):86-90.
14. Mohammed MH, Williams PA, Tverezovskaya O. Extraction of chitin from prawn shells and conversion to low molecular mass chitosan. *Food Hydrocolloids*. 2013;31(2):166-171. DOI: <https://doi.org/10.1016/j.foodhyd.2012.10.021>
15. Pavlova OV, Anufrik SS, Anuchin SN. Optimizatsiya rezhimov demineralizatsii hitinsoderzhashchego syr'ya v tekhnologii polucheniya hitozana iz micelial'nyh gribov roda Aspergillus. *Vestnik Grodnenskogo gosudarstvennogo universiteta imeni YAnki Kupaly. Seriya 6. Tekhnika*. 2017;7(1):75-82.
16. El Knidri H, Belaabed R, Addaou A, Laajeb A, Lahsini A. Extraction, chemical modification and characterization of Chitin and Chitosan. *International Journal of Biological Macromolecules*. 2018;120:1181-1189. DOI: <https://doi.org/10.1016/j.ijbiomac.2018.08.139>
17. Kavosi Rakati K, Mirzaei M, Maghsoodi S, Shahbazi A. Preparation and characterization of Poly Aniline Modified Chitosan embedded with zno-fe3o4 for cu(ii) removal from aqueous solution. *International Journal of Biological Macromolecules*. 2019;130:1025-1045. DOI: <https://doi.org/10.1016/j.ijbiomac.2019.02.033>
18. Negm NA, Hefni HHH, Abd-Elal AAA, Badr EA, Abou Kana MTH. Advancement on modification of chitosan biopolymer and its potential applications. *International Journal of Biological Macromolecules*. 2020;152:681-702. DOI: <https://doi.org/10.1016/j.ijbiomac.2020.02.196>
19. Oliver S, Vittorio C, Cirillo G, Boyer C. Enhancing the therapeutic effects of polyphenols with macromolecules. *Polym Chem*. 2016;7:1529-1544. DOI: <https://doi.org/10.1039/5PY01912E>
20. Luo Y, Wang Q. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. *Int J Biol Macromol*. 2014;64:353-367. DOI: <https://doi.org/10.1016/j.ijbiomac.2013.12.017>
21. Lee D-S, Woo J-Y, Ahn C-B, Je J-Y. Chitosan-hydroxycinnamic acid conjugates: Preparation, antioxidant and antimicrobial activity. *Food Chemistry*. 2014;148:97-104. DOI: <https://doi.org/10.1016/j.foodchem.2013.10.019>
22. Hammond MD, Skonberg I. Anti-oxidant properties of Chitosan coatings on frozenatlantic salmon fillet portions. *Journal of Aquatic Food Product Technology*. 2012;21:351-361. DOI: <https://doi.org/10.1080/10498850.2011.601536>
23. Chlitina AA, Matyushin AA. Poisk effektivnyh sorbentov putem opredeleniya ih udel'noj adsorbtsii. *The Journal of scientific articles Health and Education millennium*. 2018;20(2):84-93.
24. Kas'yanov GI, Kubenko EG. Sovremennye sposoby polucheniya i primeneniya biopolimera hitozana. *Nauka. Tekhnika. Tekhnologii (politekhicheskij vestnik)*. 2016;4:91-103.
25. Yugaj SM, Shahobutdinov SSH, Atahanov AA. IK–spektroskopicheskie issledovaniya hitozana Bombyx mori i ego proizvodnyh. *Himicheskij zhurnal Kazahstana*. 2019;3(67):254-265.
26. Hu K-J, Hu J-L, Ho K-P, Yeung K-W. Screening of fungi for chitosan producers, and copper adsorption capacity of fungal chitosan and Chitosanaceous Materials. *Carbohydrate Polymers*. 2004;58(1):45-52. DOI: <https://doi.org/10.1016/j.carbpol.2004.06.015>
27. Aydın YA, Aksoy ND. Adsorption of chromium on chitosan: Optimization, kinetics and thermodynamics. *Chemical Engineering Journal*. 2009;151(1-3):188-194. DOI: <https://doi.org/10.1016/j.cej.2009.02.010>
28. Salzano de Luna M, Altobelli R, Gioiella L, Castaldo R, Scherillo G, Filippone G. Role of polymer network and gelation kinetics on the mechanical properties and adsorption capacity of chitosan hydrogels for Dye Removal. *Journal of Polymer Science Part B: Polymer Physics*. 2017;55(24):1843-1849. DOI: <https://doi.org/10.1002/polb.24436>
29. Sakkayawong N, Thiravetyan P, Nakbanpote W. Adsorption mechanism of synthetic reactive dye wastewater by chitosan. *Journal of Colloid and Interface Science*. 2005;286(1):36-42. <https://doi.org/10.1016/j.jcis.2005.01.020>
30. Cheung WH, Szeto YS, McKay G. Intraparticle diffusion processes during acid dye adsorption onto chitosan. *Bioresource Technology*. 2007;98(15):2897-2904. DOI: <https://doi.org/10.1016/j.biortech.2006.09.045>
31. Wong YC, Szeto YS, Cheung WH, McKay G. Equilibrium studies for acid dye adsorption onto chitosan. *Langmuir*. 2003;19(19):7888-7894. DOI: <https://doi.org/10.1021/la030064y>
32. Dotto GL, Pinto LAA. Adsorption of food dyes onto chitosan: Optimization process and kinetic. *Carbohydrate Polymers*. 2011;84(1):231-238. DOI: <https://doi.org/10.1016/j.carbpol.2010.11.028>