OPTIMISATION OF CONDITIONS FOR DEACETYLATION OF CHITIN-CONTAINING RAW MATERIALS

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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 distribution coefficient in the sorbent–sorbate system 3.29·10⁻¹⁰ 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.

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→4)-linked N-acetyl-D-glucosamine. Its structure is almost identical to that of cellulose, the difference being in the presence 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, deproteinisation, 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 deproteinisation 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 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 obtaining various modifications of the sorbent 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 deacetylisation degree comparable to that of commercial chitosan [9].

Summarising all the above, chitosan can be obtained 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 deproteinisation 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 deacetylisation degree comparable to that of commercial chitosan [9].

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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 Suga Factory, Skidzyel, 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:

\[ K_d = \frac{CE}{C_{fin}} \]

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:

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

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) |
|-------------------------------------|-----------------------------|-------|-------------------------|
| Temperature, °C                     | X₁                          | Lowest (-) | 90                         | 120                        | 150 | 30                       |
| Sodium hydroxide concentration, %  | X₂                          | Medium (0) | 25                         | 30                         | 35 | 5                        |
| Exposure, min                       | X₃                          | Highest (+) | 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 |
|------------|--------------|------------------------------|
|            | X₁, t        | X₂, °C | X₃, 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:

\[ Y = 337.14 - 0.001X_1 + 0.14X_2 + 0.05X_3 \]

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.
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.

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. 5. Response surface of the chitosan adsorption activity as a function of the sodium hydroxide concentration and the temperature of treatment](image)

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

| Sample No. | Deacetylation degree, % | Specific surface area of sorbent samples, *10⁵ m²/g | Distribution coefficient in the sorbent–sorbate system, *10⁻³ cm³/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          | 42.3                    | 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                                          | 2.51                                             |
| 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                                             |
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 C\(_1\)-H bond in \(\beta\)-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/dm\(^3\), and the exposure 45 minutes: the specific surface area of the 27 sorbent samples ranged from 0.13·10\(^3\) to 0.52·10\(^3\) m\(^2\)/g, and the distribution coefficient in the sorbent–sorbate system ranged from 2.45·10\(^3\) to 3.29·10\(^3\) cm\(^3\)/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/dm\(^3\), and the exposure 45 minutes: the specific surface of the sorbent samples was 0.52·10\(^3\) m\(^2\)/g, the distribution coefficient in the sorbent–sorbate system was 3.29·10\(^3\) 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 activity 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 C\(_1\)-H bond in \(\beta\)-sugars). This sample is equivalent to the reference chitosan, the spectrum of which is taken from the IR-Spectrum library.

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