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Changes in structure of dibutyrylchitin fibres in the process of chitin regeneration

Summary — Dibutyrylchitin (DBCH) fibres were formed from the solution of the polymer in and anhydrous ethyl alcohol. The fibres obtained were then treated with a 5 % aq. potassium hydroxide solution at temperatures ranging from 20 °C to 90 °C. The process of chitin regeneration was carried out gradually and its rate depended on the temperature. High temperature alkaline treatment causes that the transformation of DBCH fibres into fibres of regenerated chitin becomes more effective. Molecular and supermolecular structural changes of fibres were examined by wide angle X-ray diffraction (WAXS), FT-IR spectroscopy and Raman spectroscopy. The obtained diffractograms were analyzed using Hindeleh and Johnson method and the computer programme "Optifit" in which Rosenbrock method was applied. The analysis of WAXS diffraction patterns showed that the curve obtained for regenerated chitin is similar to the curve obtained for the native krill chitin. The analysis of FT-IR spectra proved that as a result of hydrolysis of DBCH fibres the transformation of DBCH into chitin took place and chemical structure of the obtained chitin was almost the same as the structure of the initial native chitin.
Key words: chitin, regeneration, dibutyrylchitin fibres, supermolecular structure, WAXS, FT-IR, Raman spectroscopy.

Chitin, a polymer abundant by naturally occurring, is characterized by low solubility in organic solvents which reduces its usability. On the other hand one of well-known chitin derivatives, namely dibutyrylchitin (DBCH), is soluble in many popular solvents and thus its processing is much easier. It is possible to obtain films, fibres, microspheres *etc*. from DBCH [1]. Our interest focused on regeneration process of chitin from DBCH after alkaline treatment and molecular and supermolecular structural changes of fibres.

EXPERIMENTAL

Materials

Dibutyrylchitin (DBCH) was synthesied in the Department of Physical Chemistry of Polymers, Technical University of Łódź (Poland) by esterification of krill chitin (KCH, product of Sea Fisheries Institute, Gdynia, Poland) using butyric anhydride (Aldrich) or

perchloric acids ($HClO_4$, 70—72 %, Merck) as catalysts [2].

Ethyl alcohol and KOH (both POCh S.A. Gliwice, Poland) were used as a solvent and alkaline treatment of DBCH, respectively.

Preparation of fibres

In this study dibutyrylchitin prepared as above was next dissolved in an anhydrous ethyl alcohol and the concentrated (15 %, 18 %, 20 %, 22 %, 25 %) solution was prepared. Due to the application of five various polymer concentrations in the spinning solutions it was possible to obtain fibres having various linear densities. The total deaeration after heating of the obtained homogeneous spinning solutions was possible without the necessity of their additional filtration.

The use of ethyl alcohol as a solvent made possible elimination of a toxic action of acetone or dimethylformamide applied up to now [1]. Fibres of DBCH were spun using a wet-dry method. The fibres partly solidified, were then introduced into the water bath and taken up on a bobbin device, stretched twice and next dried in an air [3].

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This procedure was used for all the prepared spinning solutions and as a result, DBCH fibres of various diameters *i.e.*, 35, 50, 55, 67 and 75 µm were obtained.

Alkaline treatment of DBCH fibres

In the next stage of the investigation, DBCH fibres were subjected to the alkaline treatment with 1.25 M (5 %) KOH aq. solution at the temperatures of 20, 50, 70 and 90 ^{o}C (series A, B, C and D, respectively) during 10—960 min which caused their gradual transition to the regenerated chitin fibres.

Methods

X-ray diffraction

The measurements of wide angle X-ray diffraction (WAXS) for all the examined samples were carried out in the reflection mode at a room temperature using a Seifert URD-6 diffractometer with a scintillation counter connected to a computer. Ni-filtered Cu Kα radiation was applied. The accelerating voltage value was 40 kV and the plate current intensity — 30 mA. The diffraction scans were collected between 2Θ values from 2° to 60° with a step of 0.1^{o}.

WAXS investigation allows determining the following characteristics of a polymer: degree of crystallinity, dimensions of the crystallites and interplannar distances for the crystallites. The diffraction patterns obtained were then analyzed applying the Hindeleh—Johnson method [4]. The principle of the method is constructing a theoretical curve by summing peak values representing diffraction in crystalline and amorphous zones, and the background curve. The diffraction patterns were processing using the computer programme "Optifit" [5, 6] employing the Rosenbrock's method [7] in minimization. The crystalline zones' sizes were estimated by measuring a half width of a diffraction peak. The method is based on the Scherrer's formula [8].

FT-IR

All the measurements were carried out using FT-IR spectrometer of MAGNA 860 type, a product of NICOLET. The fibres were cut into 1—3 mm pieces and next they were blended with powdered sodium chloride (spectr. grade). The samples were then pressed under 200 MPa to obtain the tablets. The tablet was placed in a measuring chamber of the spectrometer equipped with a mirror bean collimator (focused 16×). As a reference, a tablet made of NaCl (without fibres) was used. The following measuring parameters were applied: range 4000—400 cm^{-1}, resolution — 4 cm^{-1}, number of scans — 128, source of radiation — IR, detector — DTGS, beamsplitter — KBr.

FT Raman

All the measurements were carried out using FT-IR spectrometer of MAGNA 860 type equipped with module FT Raman, a product of NICOLET. The following measuring parameters were applied: range 4000—100 cm^{-1}, resolution — 8 cm^{-1}, number of scans — 50 000, source of radiation — NdYag 1064 nm, energy source — 0.6 W, detector — InGaAs, beamsplitter — KBr.

RESULTS AND DISCUSSION

It was found that the hydrolysis of fibres mainly depended on their diameters. The duration of the process was from several minutes to several hours. At first the external layer of a fibre is subjected to hydrolysis. Already after a few seconds of hydrolysis process, fibres of dibutyrylchitin were covered with a layer of regenerated chitin. Keeping fibres in the solution for a longer time leads to a deep penetration of KOH into their structure.
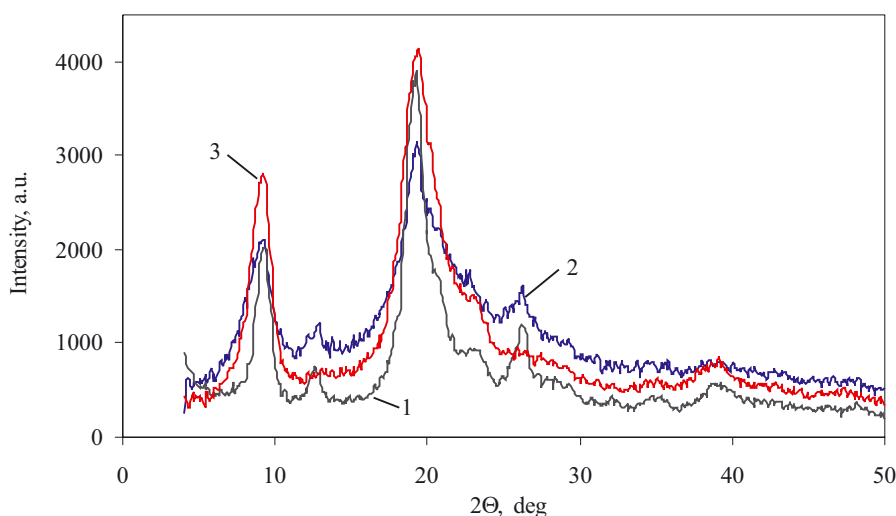

Fig. 1. WAXS diffraction patterns of KCH (curve 1) and regenerated chitins obtained using 5 % KOH (curve 2) and NaOH (curve 3) aq. solutions
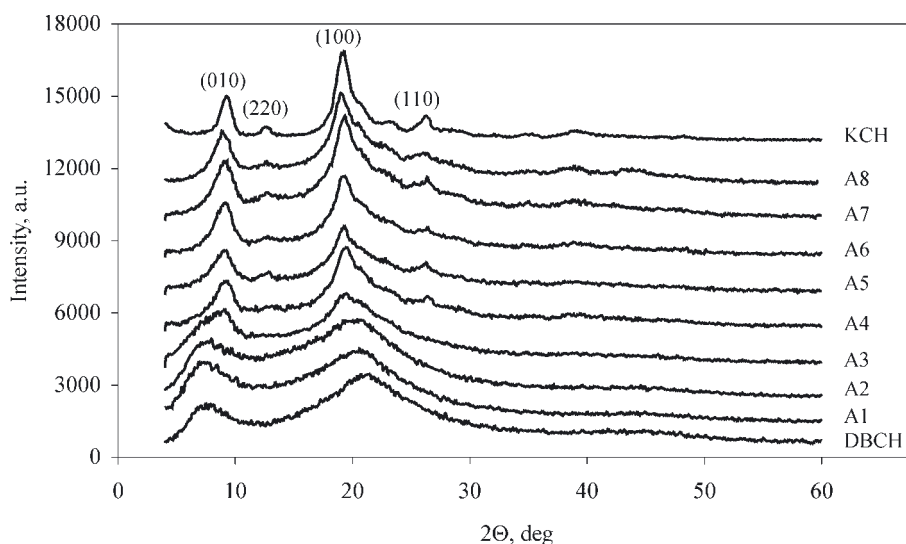
Fig. 2. WAXS diffractions patterns of KCH, DBCH fibres and products of alkaline hydrolysis of DBCH fibres at 20 °C (series A); treatment time: A1 — 10 min, A2 — 20 min, A3 — 30 min, A4 — 60 min, A5 — 120 min, A6 — 240 min, A7 — 480 min, A8 — 960 min
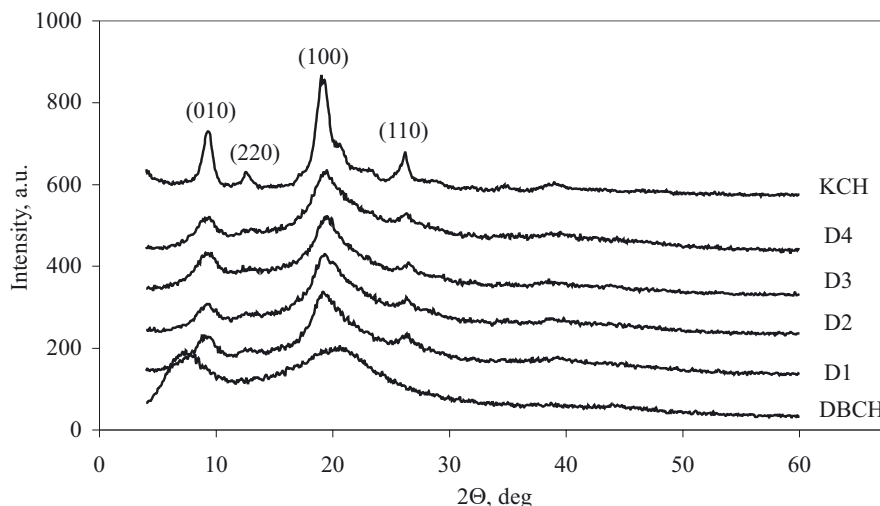

Fig. 3. WAXS diffraction patterns of KCH, DBCH fibres and products of alkaline hydrolysis of DBCH fibres at 90 °C (series D); treatment time: D1 — 10 min, D2 — 20 min, D3 — 30 min, D4 — 60 min

Deesterification of DBCH fibres to the regenerated chitin carried out by KOH aq. solutions leads to the better reconstruction of the supermolecular structure of chitin than in the case of the application of NaOH (Fig. 1). The observation confirms the earlier data given in [9—12].

Already after 120 minutes of the reaction carried out at 20 °C the shape of WAXS curve is very closed to the curve obtained for the native krill chitin.

The alkaline treatment of DBCH fibres causes chemical changes along the polymer chain, consisting in gradual breaking pendant groups off the chain. In the case of DBCH fibres transformed into the regenerated chitin, it takes place in a solid state. The process is significantly affected by such factors as the reaction environment, temperature, initial molecular and supermolecular structure morphology of treated fibres, *etc*. The applica-

tion of WAXS method allows to record diffractograms (Fig. 2 and 3) from both surface and core, yielding averaged results within the entire fibre volume.

For this reason, the interpretation of the WAXS examination (Table 1 and 2) can be regarded as an averaged result of the effects occurring within the fibre volume, possibly with a contribution from defects in the ordered areas. The biggest changes during deesterification are observed for reflection (010) when $2\Theta = 20°$. The interplanar distance calculated basing on the above reflex gradually approaches the corresponding distance for KCH in the successive stages of the alkaline treatment.

In DBCH fibres no other reflexes are observed. In deesterification series A, B, C and D only the period of reaching the interplanar distance in the perpendicular direction (010) becomes shorter which makes the polymer similar to KCH. The remaining interplanar dis-

T a b l e 1. Results of WAXS examination of DBCH fibres, krill chitin (KCH) and products of DBCH fibres alkaline hydrolysis at temperature of 20 °C (series A)

Sample	Treatment time, min	Degree of crystallinity %	Crystallite dimensions at reflection (in nm)				Interplanar distance at reflection (in nm)			
			(010)	(220)	(100)	(110)	(010)	(220)	(100)	(110)
DBCH	—	32	2.0	—	0.91	—	1.20	—	0,43	—
A1	10	37	2.2	—	1.4	—	1.20	—	0,43	—
A2	20	37	2.1	—	1.4	—	1.17	—	0,43	—
A3	30	44	1.9	3.2	2.7	—	1.08	0,66	0,46	—
A4	60	47	4.1	5.9	4.7	5.2	0.98	0.69	0.46	0.34
A5	120	62	4.2	7.5	6.8	4.2	0.99	0.70	0.46	0.34
A6	240	72	4.7	5.8	5.2	4.8	0.98	0.69	0.46	0.34
A7	480	74	4.5	4.9	4.6	4.0	0.98	0.69	0.46	0.34
A8	960	77	4.7	4.3	4.6	4.9	0.99	0.69	0.46	0.34
KCH	—	78	6.9	7.8	5.2	6.3	0.96	0.69	0.46	0.34

T a b l e 2. Results of WAXS examination of DBCH fibres, krill chitin (KCH) and products of DBCH fibres alkaline hydrolysis at temperature of 90 °C (series D)

Sample	Treatment time, min	Degree of crystallinity %	Crystallite dimensions at reflection (in nm)				Interplanar distance at reflection (in nm)			
			(010)	(220)	(100)	(110)	(010)	(220)	(100)	(110)
DBCH	—	32	2.0	—	0.91	—	1.20	—	0.43	—
D1	10	68	3.9	4.9	3.8	4.9	0.97	0.70	0.46	0.34
D2	20	72	4.3	3.9	4.2	6.5	0.96	0.71	0.46	0.34
D3	30	74	3.9	3.0	3.8	5.5	0.96	0.71	0.46	0.34
D4	60	76	4.0	3.4	3.8	5.3	0.97	0.70	0.46	0.34
KCH	—	78	6.9	7.8	5.2	6.3	0.96	0.69	0.46	0.34
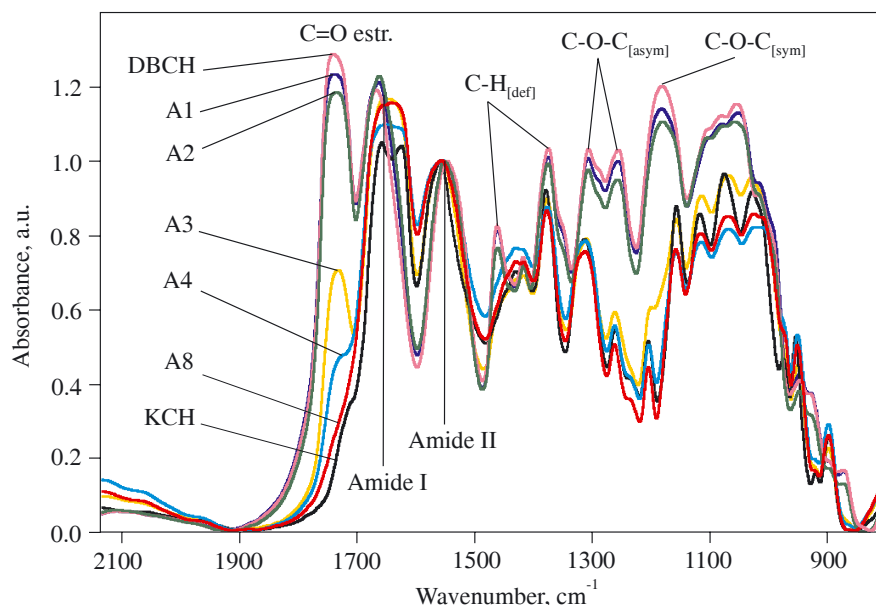
Fig. 4. FT-IR spectra of DBCH fibres, KCH and products of alkaline hydrolysis of DBCH at 20 °C (series A). For symbols see Fig. 2. Amide I: 80 % C=O, 10 % CN, 10 % NH; Amide II: 60 % NH, 40 % CN

tances directed perpendicularly (220), (100), (110) in all four series (A, B, C, and D) reach the values characteristic for KCH.

The interplanar distance calculated on the basis of this reflection gradually approaches, in the successive stages of the alkaline treatment, the distance calculated

for the krill chitin. These changes are the result of the breaking butyric substituents off the chain. During deesterification, a gradual macromolecular ordering takes place due to the structure of DBCH approaches the crystalline structure of the krill chitin. In each deestrification series, the calculated. The dimensions of the or-
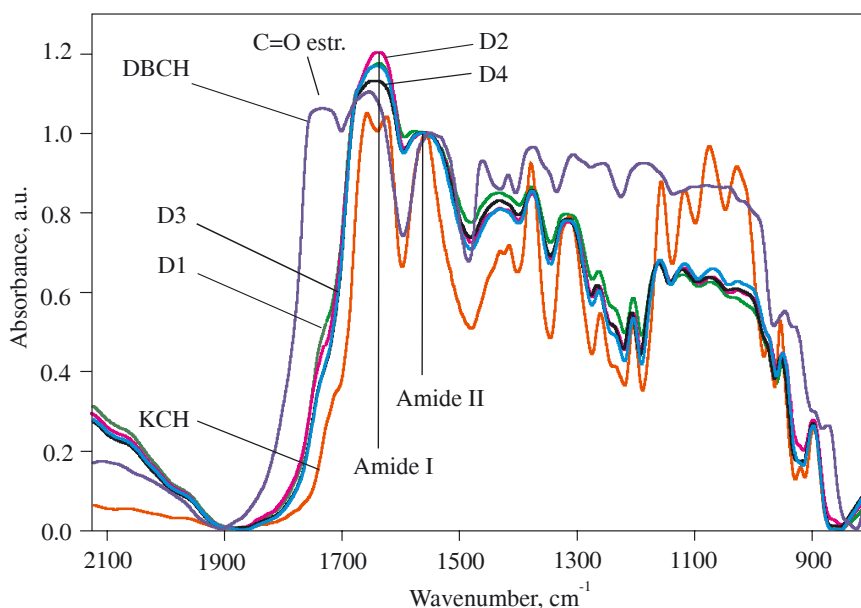
Fig. 5. FT-IR spectra of DBCH fibres, KCH and products of alkaline hydrolysis of DBCH at 90 °C (series D); for symbols see Fig. 3 and 4
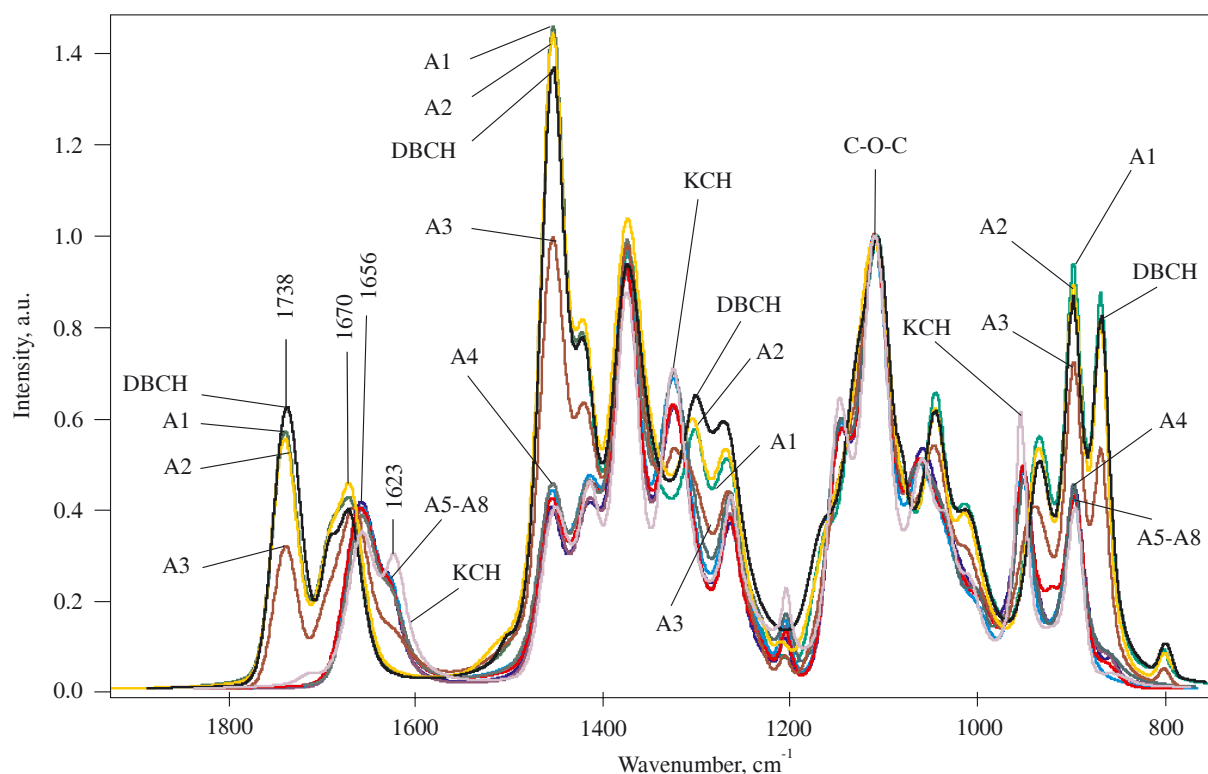

Fig. 6. FT Raman spectra of DBCH fibres, KCH and products of alkaline hydrolysis of DBCH at 20 °C (series A); for symbols see Fig. 2

dered areas calculated on the basis of half—width of crystalline reflections do not reach for any samples the values referring to the krill chitin. In each deesterification series, the calculated degree of crystalinity is close to that of KCH.

Medium IR (wave numbers = 4000—400 cm^{-1}) (Figs. 4, 5) spectra have been compared for the fibres pressed in NaCl, which were obtained during deesterification of DBCH fibres in 5 % KOH aq. solutions at 20 °C and at 90 °C. The spectrum of DBCH fibres contains an intense vibration band of strong absorption at 1740 cm^{-1} and around 1450 cm^{-1}, characteristic for the esters of fatty acids. Furthermore, bands of stronger absorption appears at around 2900 cm^{-1}, 790 cm^{-1} and 740 cm^{-1} corresponding to aliphatic groups -CH$_2$- and -CH$_3$, which content in DBCH is much higher than in chitin.

DBCH fibres spectrum contains the band from C=O oscillator of ester group. This band is the chief parameter for determining the degree of chitin esterification. In the spectra of fibres the maximum of the band drifts towards lower wave numbers, *i.e.* from 1741 cm^{-1} for DBCH, 1738 cm^{-1} for A1, 1736 cm^{-1} for A2 and 1733 cm^{-1} for A3 (Fig. 4). This drift reveals increasing action on the C=O bond energy. The band of Amide I is sensitive to chemical structure of the macromolecule. The separation of macromolecules by means of aliphatic chains of butyric acid causes breaking of hydrogen bonds formed by C=O oscillator of amide group. Oscillator C=O absorbs IR radiation for DBCH at about 1668 cm^{-1}. During the alkaline treatment of DBCH the loss of butyric substituents takes place which causes lowering of a distance between chains of macromolecules. Due to this phenomenon the C=O oscillator approaches the area of hydrogen bonds. The maximum of absorption of Amide I band drifts towards lower energy, *e.g.* for the sample A2 (in which still significant number of butyric substituents exists), to 1662 cm^{-1}. Next, a successive formation of hydrogen bonds takes place between C=O and O-H oscillators of neighbouring macromolecules. As a result of this process the defects of crystalline network affecting the supermolecular structure are fixed in the material of the fibre.

Raman spectra (Fig. 6) show that the proportions of bands for C-O-C vibrations in the heterocyclic ring and oxygen bridges do not change in time. Breaking off the butyric substituents changes the chain conformation. Hydrogen bonds appear, due to which the rings are kept within chitin planes. The conformation of the polymer chain changes rapidly when the substituents disappear, as they cause spatial disturbances in the intermolecular distances. The alkaline treatment allows the macromolecules to move closer to each other so hydrogen bonds between amide and hydroxyl groups can be formed. This allows fixing the supermolecular structure with a different polymer chain conformation after its treatement. The arrangement of the supermolecular structure was estimated by observing the behavior of the band of C-O-C vibrations in a monomer unit, C-O-C vibrations in an oxygen bridge and C-O vibrations in hydroxyl groups. The regenerated chitin spectra in this area reveal low, wide maxima. For the krill chitin, these maxima are sharp, narrow and clear. This suggests that most vibrations have the same frequency, which may happen only in an ordered material. WAXS examination confirms these observations.

CONCLUSIONS

Non-toxic fibres from regenerated chitin were obtained due to the application of ethyl alcohol as a solvent for DBCH.

The alkaline treatment of DBCH fibres results in the decreased number of the butyric groups.

Chitin regeneration process was carried out gradually, and its rate depended on the concentration of KOH and on the temperature.

The molecular structure of the obtained regenerated chitin examined by means of FT-IR corresponds to the structure of the initial krill chitin.

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