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Studies on the Biodegradation of Microcrystalline Chitosan in Aqueous Medium

Abstract

Microcrystalline chitosan (MCCh), obtained from standard chitosan of shrimp-shell origin in film form and a lyophilisate, was subjected to biodegradation in an aqueous medium. The aim of the investigation was to determine the effect of the form of the material, the time and the temperature of biodegradation on its course. The estimation of the biodecomposition degree was carried out by applying such methods as gravimetry - weight loss investigations, gel permeation chromatography (GPC) - changes in molecular structure, and FTIR - spectrophotometry. The results obtained lead to the conclusion that MCCh is a polymer which easily undergoes biodegradation. Within the range of the temperatures used in tests the best results were received at 40°C.

Key words: *microcrystalline chitosan, biodegradation, aqueous medium, analytical methods, GPC, optimisation.*

Introduction

Chitin - poly-[β-(1→4)-2-acetamido-2-deoxy-D-glucopyranose] is, after cellulose, the most abundant natural polymer. It can be found in the shells of crustaceans, insects, cell walls of micro-organisms, and also in the tissues of some fungi [1,2].

The basic obstacle to the practical utilisation of chitin is its infusibility and weak solubility, which makes it very difficult to process this polymer. One of the most important and well-known derivatives of chitin is chitosan - poly-[β-(1→4)-2-amino-2-deoxy-D-glucopyranose]. Chitosan is a product of chitin deacetylation; the most valuable property of this polymer is its solubility in aqueous solutions of organic acids.

Chitosan is used for many different applications, considering its fibre- and film-forming properties [3]; ability to form chelates with ions of heavy metals, ability to coagulate proteins, bioactivity, biodegradability, non-toxicity and also its antibacterial, antifungal and antiviral properties [1,2,4-7]. A further enlargement of chitosan applications was brought about by the invention of its new form, the so-called microcrystalline chitosan (MCCh) [8,9].

MCCh presents many advantages, such as an ability to form films directly from water suspension, good miscibility with many other polymeric materials, a high water retention value, ability to form chelates with ions of heavy metals, bioactivity, non-toxicity, antibacterial and antifungal properties, and many others.

The main purpose of this paper was to determine the effect of time and temperature of biodegradation, as well as the inoculum used and the form of the sample, on the course of MCCh decomposition carried out in the laboratory, but under conditions very close to natural ones. These studies also formed the basis for the evaluation of analytical methods which were elaborated and adjusted for the needs of this investigation. These mainly include gravimetry - loss of weight investigations, gel permeation chromatography (GPC) - changes in molecular parameters, and FTIR - spectrophotometry, and also photographic documentation of the changes.

Experimental

The MCCh samples (Table 1), coded as F (film) and L (lyophilisate), were prepared from a gel-like suspension of microcrystalline chitosan (MCCh), obtained at the Institute of Chemical Fibres, Łódź, Poland [8] from initial chitosan in the form of flakes produced by Chemopol (India), and characterised by the following properties: $\bar{M}v$=473 kD, DD=82.7%, WRV=88%, polymer content 89.70%.

The films were prepared from the suspension by drying it on Teflon plates at a tem-

Table 1. *Some physico-chemical properties of different forms of used MCCh ($\bar{M}v$ - viscometrically average molecular weight, DD - deacetylation degree, WRV - water retention value).*

Code of sample	Form of sample	$\bar{M}v$ kD	DD %	WRV %	Polymer content %
MCCh - G	gel-like suspension	440	82.7	1000	3.30
MCCh - F	film (thickness 0.14 mm)	440	82.7	172	89.04
MCCh - L	lyophilisate powder (granulation 1-10 μm)	438	82.7	486	86.30

perature of 20-22°C. Lyophilisate was obtained by sublimation drying of a frozen MCCh dispersion. The dried material was disintegrated by means of a laboratory mill.

Analytical methods

- The average molecular weight of chitosan ($\bar{M}v$) was determined viscometrically from its intrinsic viscosity value [η] at 25°C. As a solvent, the mixture containing 0.2 M of acetic acid, 0.1 M of sodium chloride and 4 M of urea in 1 cm^3 of aqueous solution was used. The value of $\bar{M}v$ was calculated according to the Mark-Houwink equation, i.e.:

  $[\eta] = 8.93 \cdot 10^{-4} \cdot \bar{M}v^{0.71}$ [10,11].

- The molecular parameters of chitosan by the GPC method were calculated on the basis of the universal calibration method using *K* and *a* values from the Mark-Houwink equation for chitosan with the deacetylation degree of 80% [12]. The equipment applied for GPC studies consisted of an HP 1050 modular liquid chromatograph (Hewlett-Packard, Germany) with a RI detector HP 1047 A, PL-GPC 4000 A and PL-GPC 300 A columns; injection volume was 70 µm, flow rate - 0.8 ml/min, continuous phase 0.33 M CH_3COOH + 0.2 M CH_3COONa and PL CaliberTM GPC/SEC software, version 5.1 (Polymer Laboratories, Ltd., Shropshire, UK).
- FTIR spectra were recorded with a Genesis Spectrometer (Unicam, England). The KBr technique was used for sample preparation. The instrument was equipped with the ATI Mattson analytical software programme.
- The water retention value *WRV* was calculated using the formula [10]:

  $WRV = [(m_1 - m_0) : m_0] \times 100\%$

  where:

  m_1 - weight of the sample after centrifugation (g),

  m_0 - weight of the sample after drying (g).
- The deacetylation degree *DD* value of chitosan samples was determined by the potentiometric titration method according to [11].

The amine group concentration *DD* was calculated from the equation:

$$\%NH_2 = \frac{16.1 \times Vr \times f}{m}$$

where:

16.1 - constant coefficient,

Vr - volume of perchloric acid in distinct inflection (cm^3) used while titrating the solution of chitosan, minus the volume of the blank sample used for titration,

f - molarity of perchloric acid,

m - weight of chitosan sample (g).

- The polymer content *PC* in MCCh suspension was calculated from the formula:

  $PC = (m_0 : m_1) \times 100\%$

Table 2. *Weight loss (wt.%) of MCCh biodegraded in aqueous medium (*micro-organisms from waste water of cellulose plant, **micro-organisms after autoselection).*

Code of sample	Form of sample	Temperature of biodegradation, °C	Period of biodegradation (weeks)	Loss of weight, %	
				1st series*	2nd series**
MCCh - L	liophilisate	30	1	66.0	74.7
			2	87.6	94.6
			4	100.0	100.0
			5	100.0	100.0
MCCh - F	films	20	1	5.3	6.2
			2	32.4	44.2
			4	46.0	52.2
			5	55.9	77.3
MCCh - F	films	30	1	16.8	84.1
			2	45.8	91.5
			4	55.6	100.0
			5	84.5	100.0
MCCh - F	films	40	1	25.9	87.0
			2	49.4	95.4
			4	91.7	100.0
			5	100.0	100.0

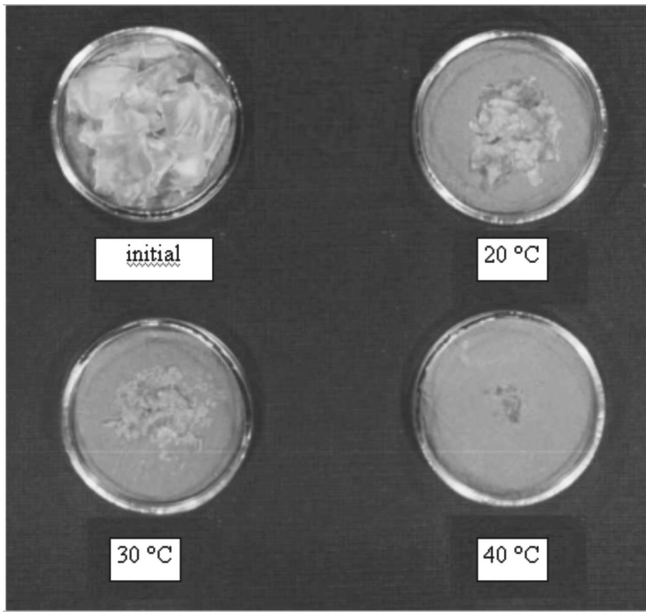

Figure 1. *Changes in samples observed during biodegradation in aqueous medium; MCCh-F after 4 weeks of biodegradation; microorganisms from waste water of cellulose plant.*

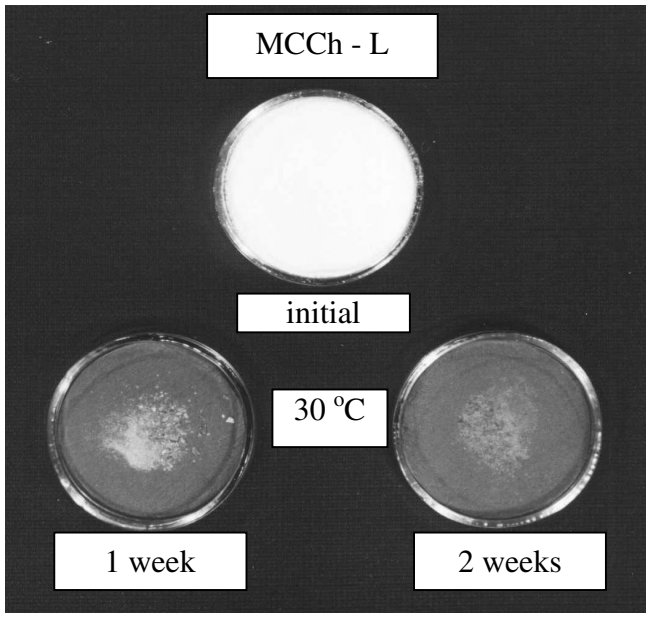

Figure 2. *Changes in samples observed during biodegradation in aqueous medium; MCCh-L biodegradation at 30°C; micro-organisms from waste water of cellulose plant.*

Table 3. *Molecular characteristic of the MCCh-F samples after biodegradation in water medium; GPC studies (*polydispersity).*

Conditions of biodegradation		DD, %	$\bar{M}_w$, kD	PD*	Fraction content, %						
temperature, °C	time, weeks				< 5	5 - 50	50 - 100	100 - 200	200 - 400	400 - 800	> 800
initial		82.3	183	6.7	3	35	19	18	14	7	4
20	1	80.2	164	6.3	4	37	19	17	13	7	3
20	2	78.5	101	5.1	4	49	19	16	8	3	1
20	4	76.6	56	4.6	11	61	14	9	4	1	0
20	5	73.5	26	3.6	22	66	7	4	1	0	0
30	1	77.9	71	7.3	15	56	12	8	6	2	1
30	2	76.3	27	3.6	20	67	8	4	1	0	0
40	1	74.7	24	3.5	23	67	6	3	1	0	0
40	2	69.4	15	3.0	33	62	4	1	0	0	0

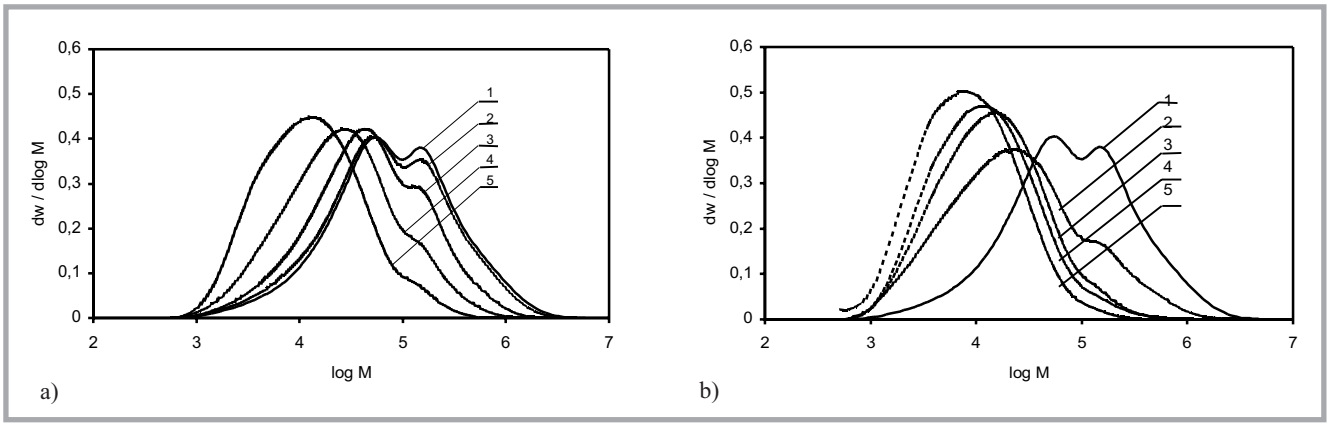

Figure 3. *Effect of time of biodegradation of MCCh-F samples: a) at 20°C: 1 - initial, 2 - 1 week, 3 - 2 weeks, 4 - 4 weeks, 5 - 5 weeks; b) at 30°C: 1 - initial, 2 - 1 week, 3 - 2 weeks, at 40°C: 4 - 1 week, 5 - 2 weeks.*

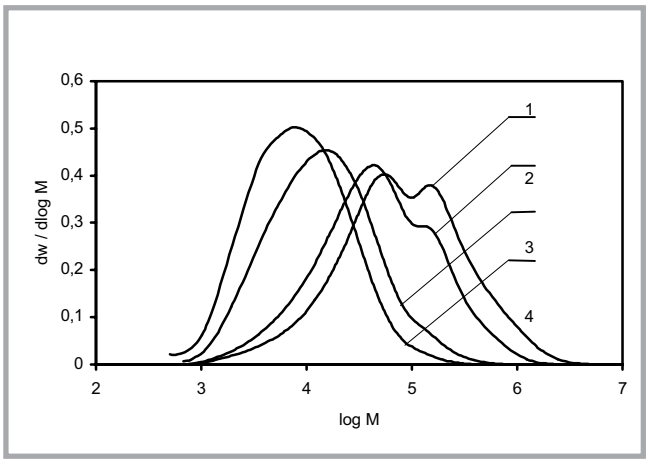

Figure 4. *Molecular weight distribution curves of the MCCh-F sample after biodegradation (time - 2 weeks):1 - initial, 2 - 20°C, 3 - 30°C, 4 - 40°C.*

where:

m_1 - weight of MCCh before drying (g),

m_0 - weight of MCCh after drying (g).

Biodegradation

The samples were subjected to biodegradation in an aqueous medium. The experiments were carried out following the procedure described in previous papers [13,14]. The biodegradation process was carried out under the conditions of carbon starvation. The reactor was filled with a bath containing all the necessary components, carbon excluded. The only carbon source in the reactor was the sample of chitosan being examined. The process in the reactor was carried out at the constant temperatures of 20, 30 and 40°C, with continuous stirring and aeration.

An active sludge from the waste water division of a cellulose plant was used as the source of micro-organisms. Due to the application of such a source, a broad spectrum of the micro-organisms occurring in nature could participate in the process, making it similar to biodegradation in a natural environment (series 1). At the same time, the fixed and repeatable conditions of the experiment were assured as described in our earlier studies [15]. During the experiment, auto-selection of the micro-organisms occurred, i.e. both spontaneous elimination of inactive ones and intensive growth of those micro-organisms for which chitosan was a good nutrient. The micro-organisms selected in such a way were applied as an inoculum in further studies (series 2).

Results and Discussion

The evaluation of the course of decomposition was carried out for MCCh films and powder (lyophilisate). Samples were removed from the bath every week. The changes were documented by taking photographs as well as by analytical methods. The purpose of these tests was to describe the changes occurring in the polymer itself. As stated above, the aim of the second series of investigation was to check whether and how using the micro-organisms after autoselection could affect the period of induction of biodegradation. The results are presented in Table 2. After biodegradation, the MCCh samples were rinsed in distilled water, then deproteinised in 0.5% NaOH for 3 hrs, rinsed again, dried to a constant weight and weighed to determine the weight loss of the sample.

The results shown in Table 2 lead to the following observations and remarks con-

cerning the course of chitosan biodegradation in aqueous solutions:

- A very long period of induction is observed (1 to 2 weeks) for the samples in film form at all the applied temperatures. At this time, the decomposition of polymer samples is relatively slow. In the third week of the process, a distinct acceleration in biodeterioration is noticed. The biodegradation undergoes the faster, the higher is the temperature of the process.
- Lyophilisate undergoes biodegradation extremely strongly. After two weeks, practically no sample residue is observed in the bath.
- The extent of the reaction increases distinctly for the samples from the second series when the biodegradation bath is enriched with the auto-selected micro-organisms strains. Thus, in order to accelerate the course of the reaction, micro-organism flora should be replenished with strains of increased bioactivity in relation to the material examined.
- The results received are illustrated very well by Figure 1 and Figure 2. Photographs were taken for the samples after the first series of biodegradation. After the second series, practically no material for further studies was obtained.

To evaluate the process of MCCh biodegradation, the gel permeation chromatography (GPC) method was used. The results are presented in Table 3 and illustrated in Figures 3 & 4. Figure 3a illustrates the molecular weight distribution (MWD) curves obtained from GPC data after 1, 2, 4 and 5 weeks of biodegradation at 20°C. Figure 3b illustrates the same relationship, but after only 1 and 2 weeks of biodegradation at 30°C; after a time longer than 2 weeks, no material for further studies could be obtained. As can be seen, at the temperature of 20°C after 1 week, only a slight MWD shift can be noted. However, after 2 weeks, a distinct shift of the MWD function towards lower molecular weight values took place. At the same time, a lowering of polydispersity M_w/M_n took place. The longer the time of biodegradation was, so a more distinct shift of MWD curves towards very low molecular weights was observed. A similar but much stronger effect was noticed for the samples biodegraded at 30°C.

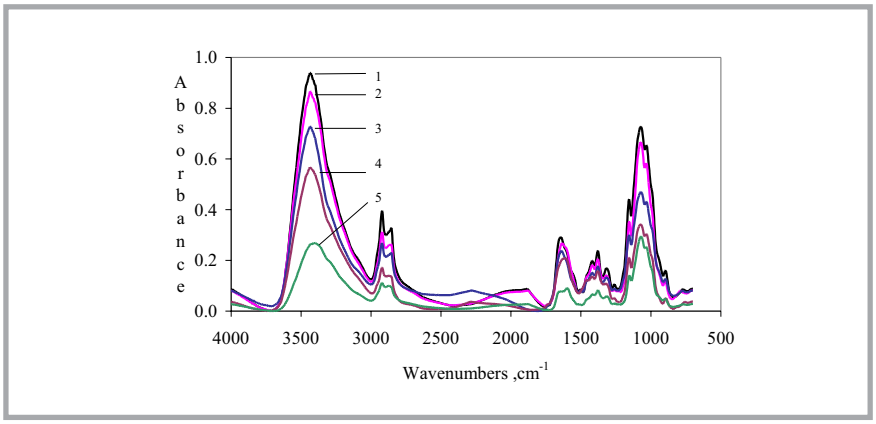

Figure 5. *FTIR spectras of MCCh-F samples after biodegradation at temperature 30°C: 1 - initial, 2 - 1 week, 3 - 2 weeks, 4 - 4 weeks, 5 - 5 weeks.*

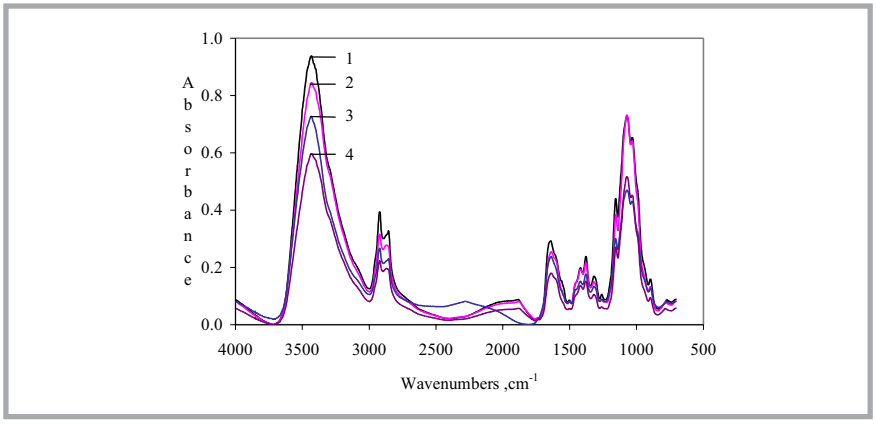

Figure 6. *FTIR spectras of MCCh-F samples after biodegradation (2 weeks): 1- initial sample, 2 - 20°C, 3 - 30°C, 4 - 40°C.*

As can be seen from the GPC results shown in Figure 4, the MCCh sample most easily biodegraded at 40°C. The MWD function distinctly shifted left towards lower $\overline{M}_w$ values. At the same time, the molecular heterogeneity parameter M_w/M_n was lowered.

The changes in the chemical structure of chitosans that occurred during biodegradation were also analysed by means of FTIR spectrophotometry. The examples of the sets of spectra are shown in Figure 5 (effect of time) and Figure 6 (effect of temperature on MCCh biodegradation). In all the cases investigated, it was demonstrated that all the bands characteristic for chitosan occur in the spectra even after five weeks of biodegradation. However, their intensities were lowered considerably . The intensity of the changes rises with the increase in the biodegradation bath's temperature. At the same the proportions between the peaks intensities are changed. The deacetylation degree (DD) calculated on the basis of spectra decreases with the progress of polymer decomposition (Table 3).

The results received for the MCCh-L sample are very close to these presented above, but the changes are much stronger. This results from the fact that the small particles of lyophilisate are much more intensively attacked by the strains collected in the biodegrading bath.

The results presented in this paper cover only a part of the experiments carried out. Results obtained for the samples of standard chitosans of different origin and different molecular weights will be reported in the next paper.

Conclusions

- The analytical methods which were applied, such as gravimetry, GPC and FTIR spectrophotometry, allow estimation of significant changes in chitosan structure caused by biodegradation.
- Based on the results obtained, it can be determined that the degradation rate rises quickly if the temperature of the process increases. The temperature of 40°C is recommended as the optimum of MCCh biodegradation in an aqueous medium.
- At temperatures in the range of 30 to 40°C of water medium, total degradation of MCCh in film form and lyophi-

lisate is observed after about 4 weeks. The period of biodegradation can be considerably accelerated by enriching a degradation bath with strains received after autoselection of micro-organisms.

- The deacetylation degree of the samples studied decreases with the progress of decomposition.

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