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Studies on the Biodegradation of Chitosan in an Aqueous Medium

Abstract
The biodegradation process of three chitosan samples characterised by similar values of deacetylation degree but different molecular weights in an aqueous medium has been studied. The biodegradation process was caused by microorganisms present in activated sludge from the waste-water treatment station of a cellulose plant. The range of the most appropriate temperatures for chitosan biodegradation was estimated as 30-36°C. The shortest time of biodegradation and the induction time was observed in the chitosan sample with the lowest molecular weight. In this article, the changes to biodegraded chitosan structure as estimated by FTIR spectrophotometry, GPC chromatography and x-ray (WAXS) methods are presented.

Key words: *chitosan, biodegradation, aqueous medium, analytical methods, FTIR spectrophotometry, GPC chromatography, x-ray methods (WAXS).*

Introduction

In the previous paper [1], some results of studies on microcrystalline chitosan (MCCh) biodegradation in an aqueous medium were presented. Two forms of MCCh samples were analysed, films and lyophilizates. The dependence of biodegradation rate on the temperature ranging from 20°C to 50°C was examined. It transpired from the above studies that total degradation of MCCh (both in the form of a film and of a lyophilizate) takes place after about 4 weeks at 30-40°C in an aqueous medium. The duration of this process can be considerably shortened if the degradation bath is enriched with bacterial strains obtained as a result of the autoselection of microorganisms.

In the present study, the evaluation of initial chitosan properties was carried out by means of respirometric tests on samples of commercial products. Weight loss determination during the process reveals the differences between the samples of various molecular weights. The changes in the molecular weight distribution (MWD) function of chitosan and the quantity of aminosaccharides liberated during biodegradation, as well as FTIR determination, make it possible to interpret the mechanism of the process. Changes in the molecular weight distribution caused by biodegradation are analysed using gel chromatography (GPC). Parameters of the supermolecular structure of chitosan samples are determined by the WAXS method.

The physico-chemical properties of chitosan are strongly affected by the molecular weight of the polymer (MW) and its degree of deacetylation (DD). In this paper, the effect of MW value on the biodegradation rate of chitosan samples has been considered. Such an assumption can be accepted provided that the effect of DD on MW determination is discounted, i.e. if the DD values of all the samples under examination are very close to each other.

Experimental

Materials and methods

In these studies, initial chitosan from shrimp shells (produced by Vanson Inc. USA) was used. Three chitosan samples coded as H (high), M (medium) and L (low), characterised by different molecular weight values and by similar degrees of deacetylation, were selected for examination of the biodegradation rate. Some properties of the initial chitosan samples in a form of flakes are presented in Table 1.

The analytical methods used in this research, i.e. molecular weight determination (viscometrically and by the GPC method) and deacetylation degree by potentiometric titration (DD) were described in detail in [1].

The FTIR spectra of chitosan samples were established in two research centres, the Institute of Chemical Fibres (IWCh)

Table 1. *Some properties of initial chitosan samples in the form of flakes (*sample specially degraded by ionising radiation, M_v - viscometrically average molecular weight, DD - deacetylation degree, WRV - water retention value).*

Sample code	$\overline{M}_w$	DD	WRV	Polymer content
	kD	%	%	%
L	97*	85.5	88	90.46
M	246	87.8	87	95.43
H	473	82.7	88	89.70

Łódź, Poland and the Institute of Textile Engineering and Polymer Materials (ITEPM), University of Bielsko-Biała, Poland. In IWCh, the FTIR spectra were recorded using a Genesis Spectrometer (Unicam, England). The KBr technique was used for the preparation of the samples. The apparatus was equipped with an ATI Mattson analytical software programme.

In ITEMP, the FTIR spectra were established using Nicolete. The FTNIR spectra within the range 6000 to 4000 cm^{-1} using the configuration of the apparatus with a calcium fluoride beam splitter and a DTGS detector were also performed.

The FTNIR spectra within the range of 10600 to 5600 cm^{-1} were established by applying the configuration of the apparatus with a calcium fluoride beam splitter equipped with an InGaAs detector.

X-ray studies were carried out using a URD6 diffractometer (Seifert). CuK_α radiation was applied, an acceleration voltage of 40 kV and an anode current intensity of 30 mA. The spectra were recorded at the range of reflection angles 2θ from 2 to 40 deg. As a result of the studies, two parameters (crystallinity index CrI and the size of crystallines D_{nkl}) were estimated.

Biodegradation

The chitosan samples were subjected to biodegradation in an aqueous medium. The experiments were carried out according to the procedure described in previous papers [2,3]. The reactor was filled with a bath containing all the necessary components, apart from carbon. The only carbon source in the reactor was the sample of chitosan under examination. Thus, in order to survive, the microorganisms had to begin the process of biodegradation. The process in the reactor was carried out at the constant temperature with continuous stirring and aeration.

An active sludge from the waste water division of a cellulose plant was used as the source of microorganisms. Due to the use of this type of source, a broad spectrum of microorganisms occurring in nature could participate in the process, making it similar to biodegradation in a natural environment. At the same time, the fixed and repeatable conditions of the experiment were assured as described in our earlier studies [4]. During the experiment, autoselection of the microorganisms occurred, i.e. both the spontaneous elimination of inactive ones and the intensive growth of those microorganisms for which chitosan was a good nutrient.

The respirometric tests were performed until full biodegradation of the polymer. The tests were carried out at 23, 30, 36, 40 and 50°C. The amount of carbon dioxide (CO_2) coming from the decomposition of chitosan which was liberated in the process of the microorganisms' metabolism was measured by the titration of hydroxide solution [5]. Its value was corrected with the amount of CO_2 liberated by a blank sample.

The second series of the experiments in an aqueous medium was carried out in a similar way to the respirometric tests, but instead of measuring the amount of CO_2, changes in the polymer samples occurring after a definite period of biodegradation were determined. The tests were performed in a aqueous medium at 20°C, 30°C and 40°C. The samples of the polymer were periodically drawn out from the bath, and after a standard procedure of purification, they were examined by means of the physico-chemical methods described previously [1].

Results and Discussion

Studies on respirometric tests of chitosan

The results of the respirometric tests of initial chitosan samples (L, M, and H) are

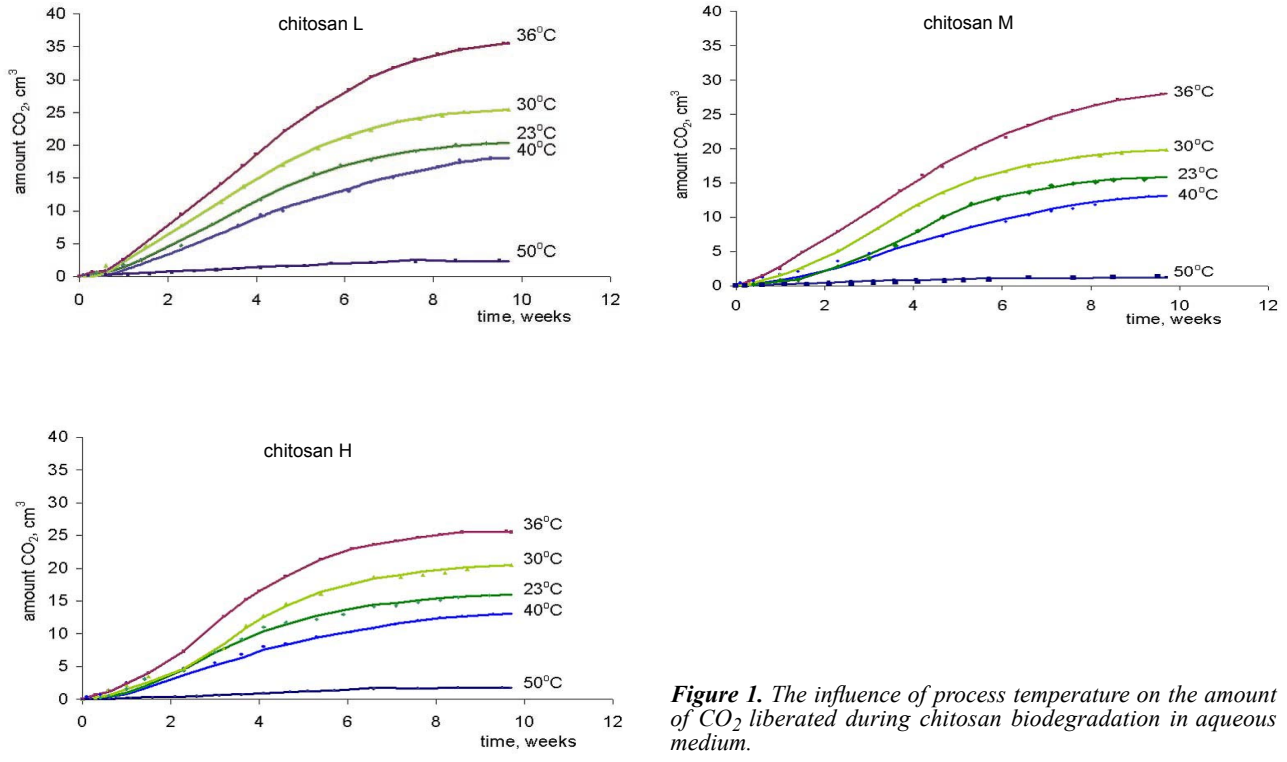

Figure 1. *The influence of process temperature on the amount of CO_2 liberated during chitosan biodegradation in aqueous medium.*

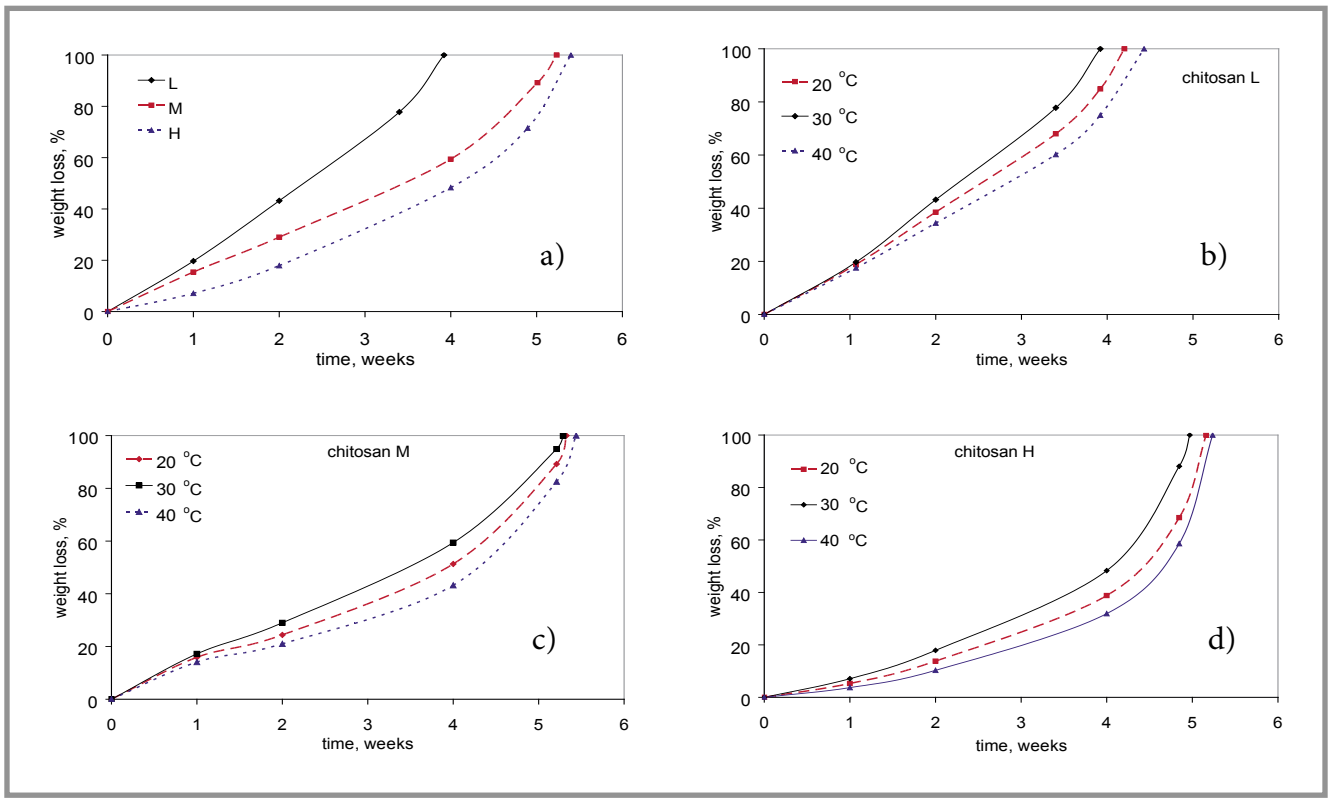

Figure 2. *Loss of weight of the chitosan samples after biodegradation: a - molecular weight dependence, temperature 30°C; b, c, d - temperature dependence, chitosan samples L, M and H.*

presented in Figure 1. The effect of temperature on biodegradation course is readily seen. Respirometric curves for the samples examined at various temperatures have a similar shape. The intensity of biodeterioration is, however, completely different. As the temperature rises, the degradation rate rises quickly. Within the range of 23-36°C, an almost double growth in the amount of CO_2 liberated at any time of biodegradation can be observed. A further increase in the temperature leads to the slowing of the biodegradation rate. At 50°C, the action of microorganisms causing the chitosan biodegradation nearly stops. Based on the results of respirometric tests, the temperature of 36°C could be indicated as those in which the process proceeds in a very good way. The decomposition of the chitosans M and H is respectively slower and very similar: chitosan L decomposes faster. This suggests that the more quickly the biodegradation proceeds, the lower is the molecular weight of chitosan, although there is no simple correlation between biodegradation rate and the molecular weight of chitosan.

Examination of changes in the structure of chitosan biodegraded in aqueous medium

The chitosan samples coded L, M and H were subjected to biodegradation in the aqueous medium at temperatures 20, 30 and 40°C. After removal from the biodegradation bath, they were washed in distilled water, then deproteinised in 0.5% NaOH for 3 hrs, washed again, dried to a constant weight at a temperature of 105°C and weighed in order to determine the weight loss of the sample. The results obtained are given in Figure 2.

The results shown in Figure 2 lead to the following observations and remarks concerning the course of chitosan biodegradation in aqueous solutions:

- For all the examined chitosan samples at all the applied temperatures, a very long period of induction is observed (1-2 weeks) during which the decomposition of polymer samples is respectively slow. At the next stage of the process, a distinct acceleration in biodeterioration is noticed. The period of induction becomes shorter at higher temperatures of biodegradation.
- The highest biodegradability was revealed by sample L, i.e. the chitosan with the lowest average molecular weight.
- The temperature of 30°C can be recommended as a good temperature for chitosan biodegradation.

Table 2. *Molecular characteristic of the chitosan M after biodegradation in aqueous medium (data from GPC studies); $\overline{M}_w$ - weight average molecular weight, M_w/M_n - polydispersity.*

Biodegradation (time of biodegradation - 2 weeks)		$\overline{M}_w$	M_w/M_n	Fraction content of $\overline{M}_w$ x 10^3, %						
		kD	-	< 5	5 - 50	50 - 100	100 - 200	200 - 400	400 - 800	> 800
Initial sample		261.1	5.7	1	25	18	23	17	9	7
Biodegradation temperature, °C	20	21.9	3.8	22	70	6	2	0	0	0
	30	7.8	2.7	50	50	0	0	0	0	0
	40	64.3	4.4	8	55	19	12	5	1	0

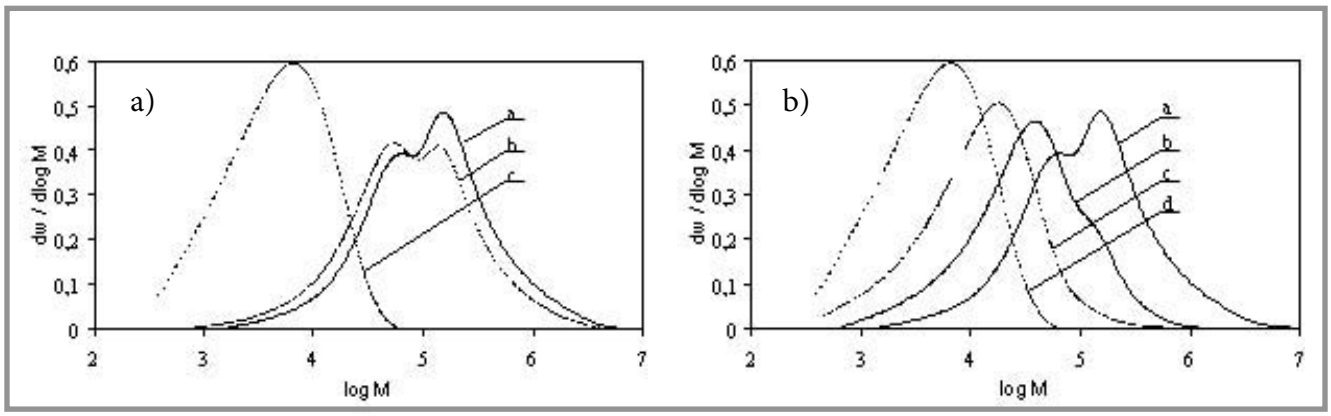

Figure 3. *Changes of the molecular weight distribution of chitosan M during its biodegradation: a) effect of time of biodegradation at 30°C, a - initial sample, b - after 1 week, c - after 2 weeks; b) effect of temperature of biodegradation, time - 2 weeks: a - initial sample, b - 40°C, c - 20°C, d - 30°C.*

As the properties of chitosan M are the most suitable from the technological point of view, further analysis were carried out on this sample alone. Figure 3a illustrates the MWD changes occurring after 1 and 2 weeks of biodegradation duration at 30°C. After 1 week, only a slight MWD displacement can be noticed, which seems to confirm the earlier observation concerning the period of induction. However, after 2 weeks, a very distinct shift of the MWD function towards a very low molecular weight values took place, as did a lowering in the polydispersity M_w/M_n. After about one week of the induction period, during the second week of biodegradation, an avalanche growth of the amount of microorganisms was observed, which caused a very strong degradation effect.

As can be seen from the GPC results shown in Figure 3b, the sample of chitosan M most easily biodegraded at 30°C, whereas at 40°C its biodegradation was most difficult. At the same time, the molecular heterogenity parameter M_w/M_n (Table 2) lowered. The molecular weight distribution (MWD) distinctly shifted left towards lower $\bar{M}_w$ values. This implies a considerable effect of temperature on the metabolism processes of bacteria in which enzymes able to attack –C-O-C- bonds in macromolecular chains of chitosan are formed.

The changes in the results caused by biodegradation are illustrated in Figure 4. In the photographs presented, it can be easily seen how the samples change under the influence of the elongated time of decomposition.

The changes in the chemical structure of chitosan that occurred during biodegradation were also analysed by means of FTIR and NIR spectrophotometry. The examples of the sets of spectra are shown in Figure 5. In all the cases investigated, it was demonstrated that all the bands characteristic for chitosan occur in the spectra even after four weeks of biodegradation. However their intensity is lowered considerably.

The lowering of the 1100 cm^{-1} and the rise of the 4930 cm^{-1} band intensity revealed by the FTMIR spectra can be interpreted as an expression of the chain lengths shortening. The analysis of the FTNIR spectra within the range 9700-5700 cm^{-1} shows that the chemical structure of polymer chains does not change noticeably. Infrared spectrophotometry at this range of wave numbers seems to have a very limited value in chitosan studies. The results obtained by x-ray studies are presented in Table 3. The results obtained indicate that the CrI rises slightly in the first stage of the biodegradation. It means that firstly, the amorphous part of the polymer is destroyed. Next, both the CrI parameters and the size of the crystallites decrease gradually with the progress of the biodegradation.

Conclusions

The results of the experiments presented in this paper were obtained from studies on the biodegradation of chitosan

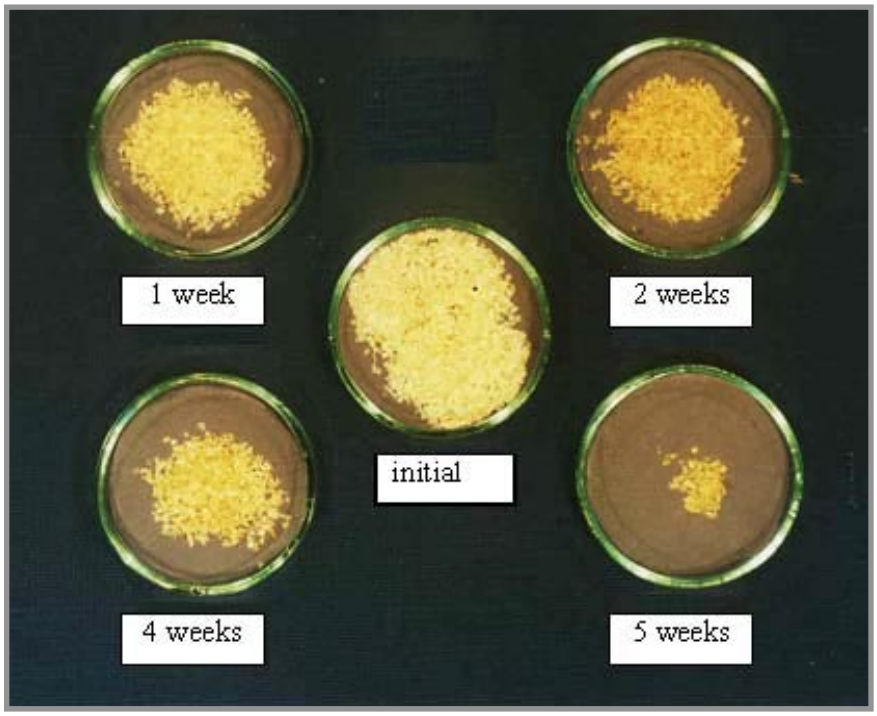

Figure 4. *Pictures in appearance of chitosan M observed during biodegradation in aqueous medium at 30°C.*

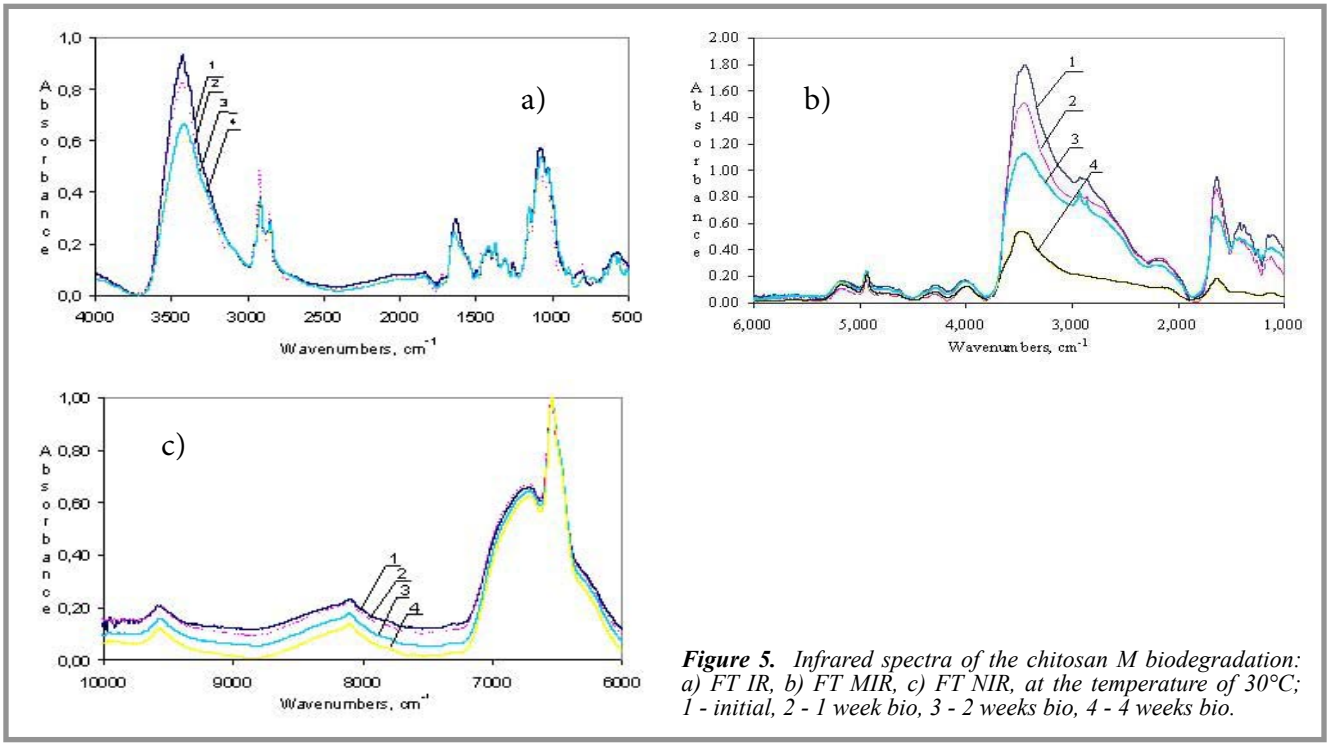

Figure 5. *Infrared spectra of the chitosan M biodegradation: a) FT IR, b) FT MIR, c) FT NIR, at the temperature of 30°C; 1 - initial, 2 - 1 week bio, 3 - 2 weeks bio, 4 - 4 weeks bio.*

in an aqueous medium. Three samples of the polymer with similar values of deacetylation degree (DD = 82-87%) but strongly differentiated molecular weight values (ranging from 97 to 473 kD) were examined.

- The process of chitosan biodegradation was monitored, among other ways, by measurements of the amount of CO_2 liberated as a result of chitosan decomposition, and also by the evaluation of structural changes to the biodegrading chitosan sample and an analysis of the amidosaccharide content in the degradation bath.
- Considering the significance of the optimum conditions for the process, respirometric tests were carried out at various temperatures ranging from 23°C to 50°C. The results obtained indicate that the biodegradation rate of tested chitosan samples is the highest at a process temperature of 36°C.
- Both respirometric tests and the determination of polymer weight loss lead to the conclusion that the biodegradation of chitosan is characterised by a relatively prolonged induction period - shorter at higher process temperatures . The sample with the smallest molecular weight was characterised by the shortest induction period.
- The results of FTIR spectrophotometry performed in two laboratories are compatible, and indicate that the range of wavelength 700-4000 cm^{-1} is the most valuable in chitosan studies. The changes observed within the range 4000-9700 cm^{-1} are very small. The FTNIR technique has only limited value in chitosan studies.
- The supermolecular structure of the chitosan changes during biodegradation process; CrI and the size of crystallites decrease gradually with the progress of process. All the other proposed analytical methods well describe the changes in chitosan structure caused by biodegradation.

Table 3. *Crystallinity index and the size of crystallites of the chitosan M samples after biodegradation (CrI - crystallinity index, D - size of crystallites).*

Biodegradation		CrI, %	D, nm		
			2θ = 10.5	2θ = 19.8	2θ = 21.9
Initial sample		43.0	4.2	5.9	4.6
Time of biodegradation	1 week	45.0	4.3	5.6	4.7
	2 weeks	45.0	4.1	5.8	4.7
	4 weeks	46.0	3.6	5.1	3.7

Acknowledgement

The investigations presented were carried out within the scope of the research project Grant No. 7 T09B 124 21 supported by the Polish State Committee for Scientific Research.

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Received 30.06.2003 Reviewed 22.09.2003