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Biodegradation of chitosan

Summary — Chitosan has been found to undergo readily to biodegradation in an aqueous medium. In this study chitosan was degraded by microorganisms from an activated waste-water sludge produced by a cellulose plant. The molecular structures of chitosan were determined by using GPC in 2, 4 and 8 weeks of treatment. Full degradation was found to have occurred in a 12-week treatment. The process of the biodegradation of chitosan can also be monitored by using IR spectrophotometry.

Controlled decomposition of synthetic polymers is a global-scale problem that poses a serious challenge to modern technology. Various solutions have been proposed involving recycling, burning, composting, and photo- (exposure to light) or biodegradation (exposure to microorganisms).

The biodegradability of a polymer depends on its macromolecular structure and the availability of a suitable microorganism. External factors such as temperature, humidity, and the type of ambient atmosphere are also of importance. Although synthetic polymers are generally highly resistant to biodegradation, some variation can be observed within this group. On the other hand, natural polymers and their modification products are more liable to microorganisms. And chitosan, a partly deacetylated chitin, is one of them.

There is little information on the biodegradability of chitin or chitosan. The available data are concerned mainly with the degradation by specific enzymes produced by certain bacteria. Chitinase, *i.e.*, the enzyme capable of decomposing chitin, was first isolated in 1971 [1]. Since then the chitin decomposing capability of chitinase isolated from some species of bacteria, has been reported [2–5]. In nature, chitinase can be found in garden and forest soil, salt marsh and elsewhere. Since the chemical structure of chitin resembles that of cellulose, a process similar to natural decomposition has been proposed [6].

At ICF, studies on chitin and chitosan were started several years ago, beginning by isolating chitin from animal tissue. Chitin was then converted into chitosan and this was further processed to yield some highly specialized products. The investigation was extended to include the degradability of chitosan, especially by selected enzymes [7, 8]. The most promising results seem to have been obtained in biomedical and agricultural applications.

The present paper sets out to describe changes in the molecular structure of chitosan due to biological decom-

position, as followed by gel permeation chromatography (GPC) and infrared (IR) spectroscopy.

EXPERIMENTAL

Commercial chitosan supplied by Pandalus A.S., Norway, a product of deacetylation of the crustacean chitin, was used.

An experimental biodegradation system was designed (Fig. 1) whose main unit was a biological reactor with a capacity of 3.5 cm³. The reactor was filled with chitosan flakes and a suitable amount of activated sludge from the waste-water treatment station of a cellulose plant was added. A standard set of nutrients, except carbon, required for the survival of microorganisms were added, chitosan being the only source of carbon in the reactor. The degradation process was carried out in an aqueous medium with continuous aeration of the system. The content of the reactor was stirred by a magnetic stirrer; temperature and pH were maintained at 24–25°C and 6.8–7.2, respectively.

The enzymes produced by microorganisms decomposed chitosan into low-molecular compounds which metabolized in air to yield carbon dioxide. This was carried away along with a stream of air and absorbed by a solution of barium hydroxide in an absorption bulb. Barium hydroxide was then titrated with hydrochloric acid to determine the amount of carbon dioxide absorbed.

Admittedly, if the enzymes are incapable of breaking up the polymer into assimilable fractions, no biodegradation takes place and the microorganisms die out for the lack of carbon.

Chitosan was sampled at 2, 4, 8, and 12-week intervals. The residual polymer was washed out and the sample was dried to constant weight and analyzed by GPC.

An HP type 1050 chromatograph was used. The GPC

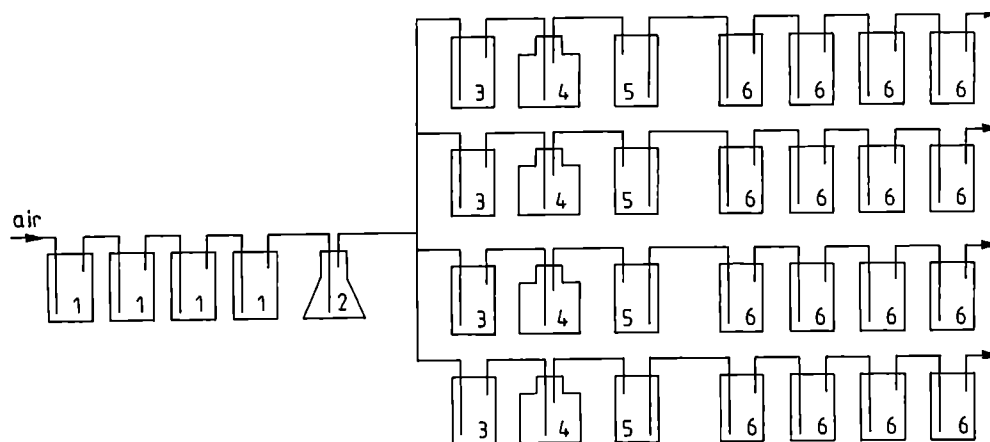


Fig. 1. Schematic diagram of the biodegradation system: 1 — NaOH, 10 N; 2 — Ba(OH)₂, 0.025 N; 3 — Empty bottle; 4 — Reactor; 5 — Empty bottle; 6 — CO₂ absorbers containing 0.025 N Ba(OH)₂

conditions were as follows: an isocratic pump HP 1050 (Hewlett-Packard); a refractometric detector HP 1047 Å (Hewlett-Packard); columns: an 8-mm PL-GFC 4000 Å and an 8-mm PL-GFC 300 Å (Polymer Laboratories, Ltd); polymer standards: polyethylene oxide (930,000—20,000), polyethylene glycol (20,000—1,470); GPC Software Version 4.0 (Polymer Laboratories, Ltd); continuous phase 0.33 M CH₃COOH + 0.2 M CH₃COONa; flow rate 1.0 mL/min; column temperature 25°C.

IR spectra were recorded with a Specord M 80 instrument (Carl Zeiss, Jena, Germany). The KBr technique was used for untreated chitosan and for the biodegraded samples.

RESULTS AND DISCUSSION

A preliminary series of experiments was carried out in order to establish optimum conditions for the biodegradation process. The bacterial strains present in the active precipitate were separated by filtration through a G2 glass funnel or by centrifugation. In one experiment the amount of bacteria was doubled.

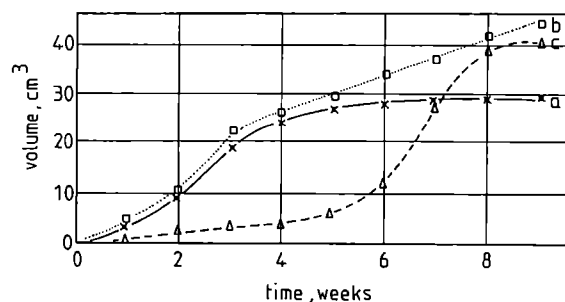


Fig. 2. Volume of CO₂ (S. T. P.) released during the biodegradation process of chitosan: a — nutrient mixture, standard concentration; b — nutrient mixture, double standard concentration; c — nutrient mixture, standard concentration, activated sludge after centrifugation and separation of active parts

Results are presented in Fig. 2. The curves *a* and *b* represent the amounts of the released carbon dioxide as a function of time at two concentration levels of the nutrient. Curve *c* represents CO₂ released on centrifugation. As can be seen from Fig. 2, a relation between the nutrient concentration and the carbon dioxide released is noticeable only in the late phase of the process. Centrifugation of the sludge has reduced the activity of the filtrate, thus slowing down the biodegradation process during the first 5–6 weeks. Then the process has rapidly accelerated as evident from curve *c*. The above preliminary results were the basis for further experiments.

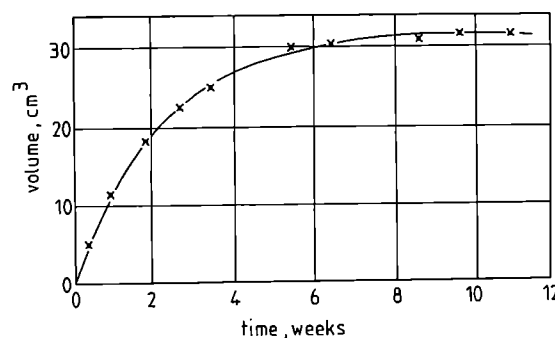


Fig. 3. Volume of CO₂ (S. T. P.) released in the biodegradation process

These are shown in Fig. 3. The curve represents the total carbon dioxide released in biodegradation under standard conditions. The points marked on the curve are the mean values of three individual experiments.

In some of our unpublished reports on commercial chitosan the polymer has been shown to degrade easily when treated by either physical or biological methods. The changes in the molecular weight distribution in chitosan caused by enzymatic treatment have been discussed elsewhere [9]. Chitosan has been suggested [9]

to alter its molecular character even during the polymer manufacturing process, as evident from the improved sorption properties of the product.

The present choice of the activated sludge from a pulp mill waste-water treatment plant was intentional. The precipitate-borne microorganisms represent various strains forming a relatively stable population. Therefore, the application of a reproducible source of microorganisms is believed to make the results obtained more representative.

Table 1 gives the average molecular weights and the ratio $\overline{M}_w/\overline{M}_n$, i.e., the polydispersity indices, of untreated and biodegraded chitosans, both determined by GPC.

Table 1. AVERAGE MOLECULAR WEIGHTS AND POLYDISPERSITY INDICES OF CHITOSAN SAMPLES

Sample	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_w/\overline{M}_n$
Untreated	83 100	444 200	5.35
Treated for 2 weeks	41 600	292 200	7.00
Treated for 4 weeks	9 700	25 100	2.60
Treated for 8 weeks	5 200	8 600	1.65

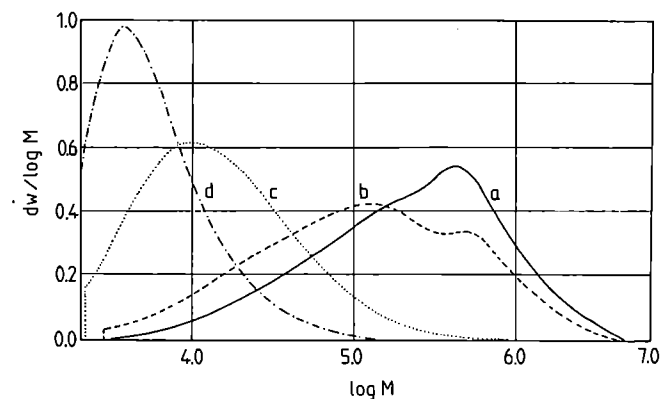


Fig. 4. Molecular weight distribution of chitosan: a — untreated; b — treated for 2 weeks; c — treated for 4 weeks; d — treated for 8 weeks

Figure 4 shows the molecular weight distribution of the *Pandalus* chitosan as supplied and after 2, 4, and 8 weeks of the treatment. In 12 weeks, the reactor was found to contain no macromolecular substances and the samples were found to have undergone some significant changes. In the 2-week treatment, the maximum shifted and the highest fraction vanished. The polydispersity, higher in 2 weeks, is explicable in terms of the statistical character of the chain fracture. As the time of treatment was protracted, the molecular weight decreased significantly and so did the polydispersity index, $\overline{M}_w/\overline{M}_n$. Two more weeks resulted in the most spectacular changes in the molecular structure of chitosan: the higher polymer fraction (over 200,000) practically disappeared and a single maximum of MWD appeared in the area of low molecular weights. This tendency strengthened as the

time of the biodegrading treatment was prolonged. In 8 weeks, the sample contained only the macromolecules less than 50,000 in molecular weight. Changes in the molecular structure of chitosan are presented in Table 2, together with the dwindling content of the higher fractions. Low-molecular-weight compounds were readily assimilated by the microorganisms in the reactor.

Table 2. CONTENT OF MACROMOLECULES WITHIN DEFINED LIMITS OF MOLECULAR WEIGHT

Sample	Molecular weight (thousands)						
	<5	5 to 50	50 to 100	100 to 200	200 to 400	400 to 800	>800
Untreated	1	15	11	17	19	23	14
Treated for 2 weeks	2	29	16	16	14	13	10
Treated for 4 weeks	17	71	8	3	1	0	0
Treated for 8 weeks	54	45	1	0	0	0	0

The present results seem to be encouraging enough to make further investigation worthwhile.

The structural changes of the decomposing chitosan can also be followed by IR spectroscopy.

IR spectra of untreated chitosan and of one degraded for 8 weeks are presented in Fig. 5. In the latter spectrum some peaks are missing, viz., those corresponding to the -COC- bonds (wavelengths 1,000–1,200 cm^{-1}), -CH₂- groups (2,000 cm^{-1}), and Amide III (superposition of -NH- and -CN at 1,310 cm^{-1}).

The extent of changes makes the IR method a very convenient tool for monitoring chitosan degradation process. The present results fully confirm our previous supposition regarding the high sensitivity of chitosan to biodegradation [10, 11]. The biodegradation rate is affected by several factors, primarily by the concentration of the nutrient and the form of the microorganisms introduced. The microorganisms from a renewable in-

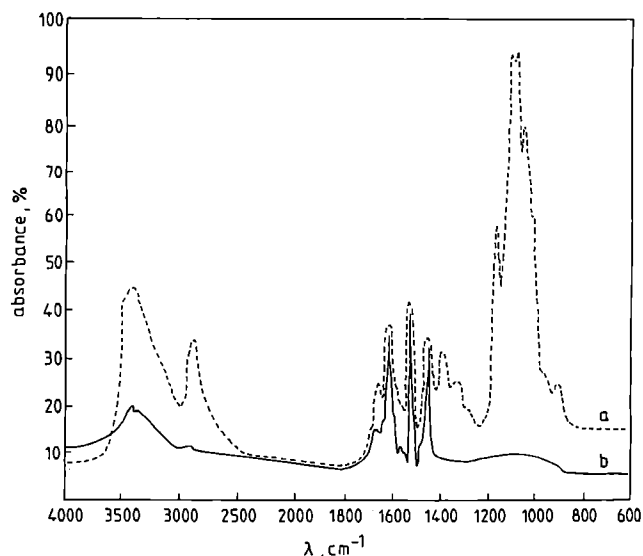


Fig. 5. IR spectra of chitosan: a — untreated; b — treated for 8 weeks

dustrial source are shown to have been capable of degrading commercial chitosan in a relatively short time in an aqueous medium. The two techniques, *i.e.*, GPC and IR spectrophotometry, are seen to lend themselves perfectly well to following the process of degradation: they will be used in our further investigations.

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