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Study on the synergetic degradation of chitosan with ultraviolet light and hydrogen peroxide

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Abstract—Chitosan was effectively degraded by hydrogen peroxide under irradiation with ultraviolet light. The existence of a synergetic effect on the degradation was demonstrated by means of viscometry. In addition, the optimal conditions of degradation were determined on the basis of orthogonal tests. The structure of the degraded product was characterized by Fourier-transform infrared spectra (FTIR) analysis and diffuse reflectance spectra (DRS) analysis. The mechanism of the degradation of chitosan was correlated with cleavage of the glycosidic bond.

Keywords: Chitosan; Degradation; Synergetic effect; Hydrogen peroxide; Ultraviolet light irradiation

1. Introduction

Chitosan, (1→4)-2-amino-2-deoxy-β-D-glucan, is a natural polymer generally obtained by extensive deacetylation of chitin isolated from crustacean shells. Chitosan and its derivatives have a special set of interesting properties: nontoxicity, biocompatibility, controllable biodegradability, and nonantigenicity.[1,2] These properties make chitosan an attractive biopolymer for applications in wide areas such as biotechnology, pharmaceuticals, wastewater treatment, cosmetics, agriculture, food science, and textiles.[3]

Generally, chitosan obtained from the deacetylation of chitin has a high molecular weight and low solubility in most solvents, which limits its applications, especially in medicine and in the food industry. However, when chitosan is degraded, its solubility can be improved. Furthermore, chitosan oligomers obtained from chitosan were shown to possess improved biological, chemical, and physical properties compared to chitosan.[4,5] Muzzarelli et al.[6] demonstrated that chitosan with an average molecular weight in the range of 1000–10,000 Da is most desirable for a number of medical and biotechnological applications. Thus the development of an efficient process for reducing the molecular size of chitosan, without altering its chemical structure, is of great interest.

Like other polysaccharides, chitosan is susceptible to a variety of degradation techniques, including acid hydrolysis,[7,8] oxidative degradation,[9,10] and enzymatic methods.[11,12] Recently, oxidative degradation with hydrogen peroxide has been studied.[13–17] The technique is based on the formation of a reactive hydroxyl radical by the disassociation of hydrogen peroxide. Hydroxyl radicals, which are powerful oxidizing species, can attack the β-D-(1→4) glucosidic linkages of chitosan. However, the disassociation of hydrogen peroxide is inefficient when hydrogen peroxide is used alone.

Chemical oxidation using hydrogen peroxide in the presence of ultraviolet radiation is a very promising technique that can be applied successfully in many oxidation processes. Ultraviolet light ($\lambda < 300$ nm) has been found to be very effective for the disassociation of hydrogen peroxide and generation of hydroxyl radicals,[18] which will undoubtedly accelerate the degradation of chitosan. In this paper, the degradation

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of chitosan with hydrogen peroxide under irradiation with ultraviolet light was studied. We demonstrated the existence of a synergetic effect on the degradation; in addition, the optimal conditions of degradation were described by an orthogonal test. The mechanism of the degradation was studied by FTIR analysis. Moreover, the effect of ultraviolet radiation time on total organic carbon (TOC) content of the degradation solutions was also investigated.

2. Experimental

2.1. Materials and apparatus

The starting materials included chitosan with degrees of acetylation of 95.54% (Yuhuan Biology Engineering, Zhejiang, China), hydrogen peroxide, acetic acid, sodium acetate, and distilled water. The photoreactor apparatus used in this study was made in our lab with low-pressure mercury lamps emitting at 254 nm. A Ubbelodhe viscosimeter and R-201 rotary evaporator were from Shanghai of China.

2.2. Characterizations

The intrinsic viscosities were determined using an Ubbelodhe viscosimeter by capillary viscometry at 30 °C. Prior to measurement of flow times, chitosan solutions were dissolved in 0.1 mol/L CH_3COONa–0.2 mol/L CH_3COOH solution. The intrinsic viscosities $[\eta]$ were calculated according to the following equation:[19]

$$[\eta] = \frac{\eta_{sp} + 3\ln\eta_r}{4c} \quad (1)$$

Here, η_r, η_{sp} refer to the relative viscosity and the incremental viscosity, respectively, and c is the concentration of chitosan (g/mL). The viscosity average molecular weight (M_v) was calculated according to the following equation:[20]

$$[\eta] = 1.81 \times 10^{-3}\ M_v^{0.93} \quad (2)$$

The IR spectra of the original chitosan and oligomers were obtained with a Nicolet Nexus 670 FTIR spectrometer using KBr pellets. Solid samples were mixed with KBr to form a homogeneous mixture for the FTIR measurements. UV–vis absorption spectra of the original chitosan and oligomers samples were recorded on a Cary-500 UV–vis–NIR spectrophotometer equipped with a HARRICK diffuse reflectance accessory.

The TOC of chitosan solutions during degrading were measured on a Shimadzu TOC-5000A analyzer. The mineralization rates of chitosan solutions during the oxidative degradation process were also calculated according to the following equation:

$$X_{MIN}(\%) = \frac{TOC_0 - TOC_x}{TOC_0} \times 100 \quad (3)$$

where X_{MIN} refers to mineralization rate, TOC_0 refers to the TOC content of the initial chitosan solution, TOC_x refers to the TOC content of chitosan degraded solution at different illumination time, respectively.

The rate of decrease in viscosity was calculated according to the following equation:

$$R\ (\%) = \frac{\eta_0 - \eta_t}{\eta_0} \times 100 \quad (4)$$

Here, R refers to the rate of decrease in viscosity, η_0 refers to the intrinsic viscosity of initial chitosan, η_t refers to the intrinsic viscosities of chitosan degraded solution at different times.

2.3. Preparation of chitosan oligomers

Chitosan 1 g was dissolved in HOAc solution under room temperature, then poured into a solution of 50 mL of 2% (w/v) H_2O_2 and 1% (w/v) HOAc. At 40 °C, the solution was irradiated for 30 min with a low-pressure mercury lamp emitting ultraviolet light at 254 nm. The surface area of solutions was 12 cm^2, and the intensity from the center of the lamp to the exterior of the reactor is 4 mW/cm^2. Solutions were stirred constantly during illumination. After reaction, the resulting solution was evaporated under low pressure with an R-201 rotary evaporator. When the volume of solution was reduced to one-fourth of the original volume, the chitosan oligomers were precipitated with acetone, washed thoroughly with acetone, then dried under vacuum at 40 °C.

3. Results and discussion

3.1. Synergetic degradation of chitosan by integrated ultraviolet radiation and hydrogen peroxide

In order to examine whether a synergetic effect that combines ultraviolet light and hydrogen peroxide is operative in the degradation of chitosan, three experiments were designed. The concentration of every reactant and correlative conditions used in the various experiments are given in Table 1.

Table 1. Reaction conditions of three degradation experiments

	Experiment number		
	1	2	3
Concentration of chitosan (w/v)	2	2	2
Concentration of acetic acid (w/v)	1	1	1
Concentration of H_2O_2 (w/v)	0	2	2
Ultraviolet radiation time (h)	3	0	3

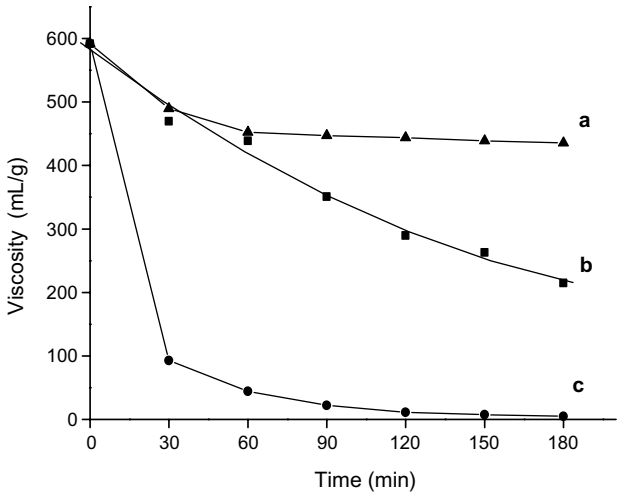

Figure 1. The intrinsic viscosities versus reaction time in the different treat-methods. (a) Irradiation alone; (b) only with hydrogen peroxide; (c) with irradiation and hydrogen peroxide oxidation.

The total volume of solution was 50 mL for each of the three experiments, and a mercury lamp emitting at 254 nm was used in experiments 1 and 3. At various intervals, the intrinsic viscosities were determined using an Ubbelodhe viscosimeter at 30 °C. The decay of the intrinsic viscosities as a function of reaction time is depicted in Figure 1.

The intrinsic viscosities of initial chitosan were 592 mL/g in all cases. When the initial chitosan was only exposed to ultraviolet irradiation [case (a) in Fig. 1], the intrinsic viscosity of chitosan was decreased to 490 and 436 mL/g for 30 and 180 min, the rate of decrease in viscosity was 17.2% and 26.4%, respectively. Under individual hydrogen peroxide oxidation [case (b) in Fig. 1], the intrinsic viscosity of chitosan was decreased to 470 and 215 mL/g in 30 and 180 min, the rate of decrease in viscosity was 20.6% and 63.7%, respectively. It is interesting to note that in the system combined with ultraviolet radiation and hydrogen peroxide oxidation [case (c) in Fig. 1], the rate of decrease in viscosity increased to 84.3% and 99.2%, which is higher than the summation of those numerical values obtained by the ultraviolet radiation and hydrogen peroxide processes acting alone, respectively. In other words, synergetic effects are operative in the degradation of chitosan with ultraviolet light and hydrogen peroxide.

3.2. Orthogonal test

The optimal conditions for degrading chitosan were studied by an orthogonal test. Four controllable variables, the concentration of chitosan, acetic acid, hydrogen peroxide, and irradiation time were selected, each at three levels. The variables that were investigated, and their test levels are listed in Table 2.

Table 2. The variables investigated and their levels

Variables investigated	Levels of each variable		
	1	2	3
A: concentration of chitosan (w/v)	1%	2%	3%
B: concentration of acetic acid (w/v)	1%	1.5%	2%
C: concentration of hydrogen peroxide (w/v)	0	2%	4%
D: irradiation time (h)	0	0.5	1.0

Table 3. Experimental arrangement and test results

Experiment number	*A*	*B*	*C*	*D*	Intrinsic viscosity (mL/g)
1	1	1	3	2	45.45
2	2	1	1	1	565.21
3	3	1	2	3	64.37
4	1	2	2	1	361.36
5	2	2	3	3	36.96
6	3	2	1	2	476.18
7	1	3	1	3	520.88
8	2	3	2	2	58.93
9	3	3	3	1	169.29
K_1	927.69	675.03	1562.27	1095.86	
K_2	661.10	874.50	484.66	580.56	
K_3	709.84	749.10	251.70	622.21	
Variance	266.59	199.47	1310.57	515.30	

Reference to the experimental design theory, the orthogonal array L_9 (3^4) was selected to arrange the test program. The test results are listed in Table 3.

Obviously, the order of influence of each variable is $C > D > A > B$. The variance of concentration of hydrogen peroxide is the greatest, and level 3 is the best according to the orthogonal test, above. The optimum level of each variable is $A_2B_1C_3D_2$. However, it should be noted that the excess of hydrogen peroxide likely acts as the scavenger of hydroxyl radicals,[21] which may reduce the reactive efficiency. Therefore 2% was chosen as an appropriate concentration of hydrogen peroxide. Thus the optimum reaction conditions were determined as follows: hydrogen peroxide 2% (w/v), irradiation time 30 min, chitosan 2% (w/v), acetic acid 1% (w/v).

3.3. FTIR spectral analyses and DRS spectral analyses

The IR spectra of original and degraded chitosan are shown in Figure 2. The main bands in the spectrum of original chitosan are as follows: Both the N–H stretching and O–H stretching vibrations can be characterized by a strong, broad band centered at 3440 cm^{-1}. The bands at 1602 and 599 cm^{-1}, correspond to the binding vibrations of the amido groups, the bands in the range 1158–895 cm^{-1} are assigned to the characteristics of its polysaccharide structure,[22] and the peaks at 2875 and 2920 cm^{-1} are due to C–H stretching. The sharp band

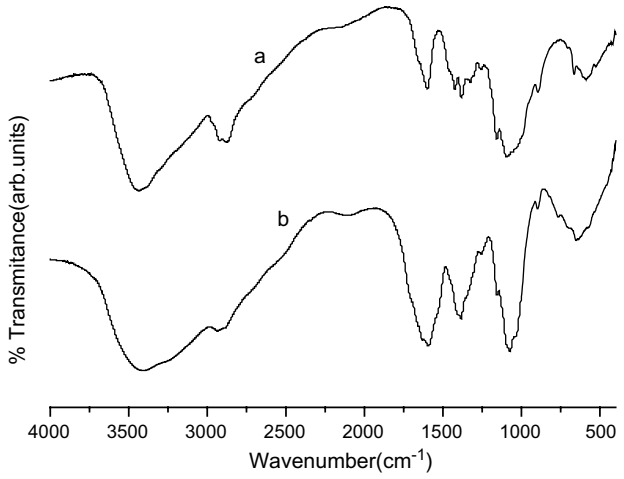

Figure 2. FTIR spectra of (a) chitosan and (b) degraded chitosan.

at 1383 cm^{-1} has been assigned to C–H bending and C–H stretching.

The spectra of degraded chitosan exhibited most of the characteristic adsorption peaks of chitosan but with some differences: the vibrational band at 1100 cm^{-1} that corresponds to the ether bond in the pyranose ring was weakened, which indicates that the rupture of the β-glycosidic bonds may have led to several effects on the amount and distribution of glycosidic bonds in the molecular chains of chitosan. The bands at 1602 and 599 cm^{-1} become stronger, and the peak at 599 cm^{-1} moved toward the higher wavenumbers. In addition, the N–H stretching vibration moved toward lower wavenumbers, which indicates that the intermolecular and intramolecular hydrogen bonds of chitosan were weakened and its crystallinity was reduced after degradation.

In order to study if the carboxylic or aldehyde groups formed after the β-D-(1→4) glycosidic bonds of chitosan were broken, the UV–vis spectroscopy of the original chitosan and degraded chitosan were carried out.

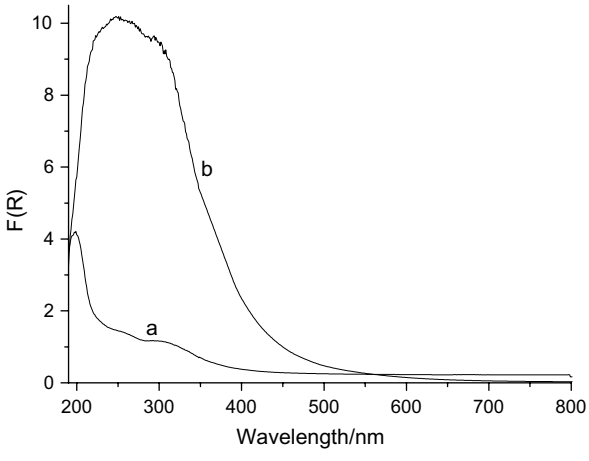

Figure 3. DRS patterns of (a) chitosan and (b) degraded chitosan.

Figure 3 shows the UV–vis spectra of (a) original and (b) degraded chitosan. For original chitosan, a strong absorption band was evident around 200 nm, which was caused by the n→σ* transition for the amido groups of chitosan.

After degradation with ultraviolet light and hydrogen peroxide, an absorption band was observed at 260 nm, which corresponds to the n→σ* transition for the amido groups. It is important to note that a spectral absorption band could be seen at 300 nm, which is assigned to the n→π* transition for the carbonyl or carboxyl groups. It might be the new side group of degraded chitosan.

3.4. Mechanistic discussion

It is obvious that carbonyl groups could be found in the chitosan oligomers via DRS analysis. Qin et al.[16] and Tian et al.[17] confirmed that treatment of chitosan with H_2O_2 resulted in the formation of carbonyl groups. Furthermore, the photodegradation of chitosan with ultraviolet light alone could produce carbonyl groups besides the cleavage of the glycosidic bond.[23]

Hydrogen peroxide has a much stronger molar absorption and the highest quantum yields at 254 nm.[24] When ultraviolet light with a wavelength of 254 nm is absorbed directly by hydrogen peroxide, the disassociation by photolysis of the –O–O– peroxide bond is accelerated, and hydroxyl radicals are effectively generated by the following equation:[25,26]

$$H_2O_2 + h\nu \rightarrow 2 \cdot OH. \quad (5)$$

Hydroxyl radicals, which are highly powerful oxidizing species,[18] can oxidize a broad range of organic compounds. Consequently hydroxyl radicals generated in the experimental systems attacked the β-D-(1→4) glycosidic bonds of chitosan and subsequently broke the glycosidic linkages. Moreover, the photodegradation of chitosan may occur during ultraviolet radiation, which served to decrease the average molecular weight of chitosan.[15]

3.5. Analysis of mineralization

Combining ultraviolet light and hydrogen peroxide oxidation has been applied successfully in many advanced oxidation processes, but it may lead to complete mineralization. Then the TOC in the degradation process was determined with the TOC-5000A analyzer, and the mineralization rate was calculated according to Eq. 3. As shown in Figure 4, the TOC decreased and the mineralization rate increased with increasing irradiation time. In the first three hours, the mineralization rate was under 5%. The results show that the degradation of chitosan by this method is feasible.

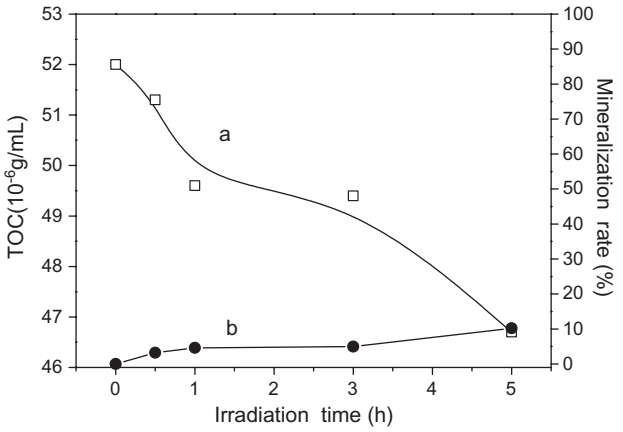

Figure 4. Effect of ultraviolet radiation time on (a) TOC and (b) mineralization rate of 50 mL of chitosan solution under the following conditions: chitosan: 2% (w/v), H_2O_2: 2% (w/v), acetic acid: 1% (w/v), illumination time: 0.5 h, 1 h, 3 h, 5 h; temperature: 40 °C.

4. Conclusions

The degradation method that combined ultraviolet light and hydrogen peroxide exhibited a synergetic effect. The optimum reaction conditions were obtained by an orthogonal test using hydrogen peroxide 2% (w/v), chitosan 2% (w/v), acetic acid 1% (w/v), and an irradiation time of 30 min.

Chitosan oligomers retained almost the backbone of the chitosan macromolecular structure; the breaking of the C–O–C glycosidic bond led to chain scission and the formation of carbonyl groups.

The mineralization rate was low in the degradation process combined with ultraviolet radiation and hydrogen peroxide oxidation. Therefore the degradation method is feasible, convenient, and potentially applicable.

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