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Ultrasonic Degradation of Polysaccharide from a Red Algae (*Porphyra yezoensis*)

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Polysaccharide from *Porphyra yezoensis* (PYPS) was degraded by ultrasound in this study. The changes of intrinsic viscosity with various conditions, such as ultrasonic power, irradiation time, reaction temperature, initial pH value of solution, and its concentration, were investigated by using Ubbleholde viscometry. It was found that the ultrasonic degradation rate of PYPS solution increases with the increase of ultrasonic power and reaction temperature and the decrease of the initial pH value of the solution. The order of the susceptibility of initial PYPS concentrations on degradation is 0.75 > 0.5 > 1.00 > 2.00 g/dL. The mechanism about the ultrasonic degradation of PYPS may be explained by more for mechanical and less for radical effects. Relationships between degradation rate and ultrasound time are exponential functions. The activation energy of ultrasonic degradation of PYPS solution is 52.13 kJ/mol, which was calculated by the logarithmic form of the Arrhenius equation, and is lower than one for the acid or enzyme catalyzing degradation of similar glycosidic bonds.

KEYWORDS: Polysaccharide; ultrasonic degradation; *Porphyra yezoensis*; intrinsic viscosity

INTRODUCTION

Recently, ultrasonic degradation of polysaccharide solutions has been extensively investigated in the laboratory and in the food industry. In the case of polysaccharide degradation, studies on the molecular properties of polysaccharides degraded by ultrasound have been investigated on starches, celluloses, dextrans, and chitosans (*1*−*9*). Otherwise, many properties of polysaccharides depend on their molecular weights. This refers to both physical characteristics such as solution viscosity and more complex properties such as bioactivity. It was confirmed that polysaccharides from *Porphyra yezoensis* (PYPS) digested by using β-agarase showed a higher macrophage stimulation activity and solubility (*10*, *11*). Similarly, antithrombotic or stimulating plant growth activities also depend on its molecular weight (*12*, *13*).

Because the average molecular weight of native polysaccharides is determined by their source of origin and cannot be, in most cases, easily degraded, it is necessary to set up a kind of fast, efficient, and convenient method of degrading the native polysaccharides in order to achieve the desired molecular weight (*14*). Chemical treatment, such as acid or alkali hydrolysis (*15*−*18*), is often used, but its consumption of solvents and energy is vast and chemical wastes are often unavoidable. So, the physical degradation method of polysaccharide is potentially likely. On the basis of the above fact, the method of ultrasonic degradation was used in this study. For investigating the variables influencing polysaccharide ultrasonic degradation, the effects of ultrasonic power, irradiation time, reaction temperature, initial pH value, and concentration of solution are studied in our work. At the same time, the purposes of this study were also to set up a kinetics equation describing the ultrasonic degradation of polysaccharide and to elucidate the mechanism about the effects of molecular weight (using intrinsic viscosity as an index) on the degradation.

P. yezoensis is an important ocean resource all over the world, and PYPS possesses many biological activities (*10*, *11*, *19*). Other researchers have proved that the PYPS digested with β-agarase showed higher macrophage stimulation activities and solubilities than the native one (*11*). So, the PYPS was used as a sample for investigating ultrasonic degradation of polysaccharide in this study.

MATERIALS AND METHODS

Samples. *P. yezoensis* was purchased from Nantong Lanbo Industry Co. Ltd. (Jiangsu, China). *P. yezoensis* powder (40 mesh sieve) was defatted with supercritical CO_2 for 5 h under the following conditions: extraction, 45 °C, 30 MPa; separation, 35 °C, 5 MPa; and CO_2 flow rate, 10 L/h. A 116 g amount of the defatted *P. yezoensis* was extracted with 10 L of distilled water at 100 °C for 4.7 h. Then, the extract was centrifuged at 3500 rpm for 15 min, the supernatant was filtered with Whatman no. 1 paper (Shanghai, China), and the filtrate was condensed to about 100 mL. Ethanol (400 mL, 95% v/v) was added to the condensed filtrate and left overnight at 4 °C. After the resulting precipitate had been collected by centrifugation (10 min, 3000 rpm), it was dispersed with 100 mL of water and subjected to the Sevag method to remove free proteins (*20*). The extract was ultrafiltered (M_w cutoff, 10 kDa; PLCC 10K regenerated cellulose, Millipore), and the retentate was precipitated in 95% ethanol of four times volume. Then, the precipitate collected by centrifugation was dried at 45 °C in vacuo; thus, the PYPS was obtained.

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Table 1. Effects of Ultrasonic Power, Initial Solution Concentration, Initial Solution pH, and Temperature on the Ultrasonic Degradation of PYPS

experimental group[a]	G1	G2	G3	G4
ultrasonic power (W)	400, 800, 1000, 1200	800	800	800
initial solution concentration (g/dL)	1.00	2.00, 1.00, 0.75, 0.5	1.00	1.00
initial solution pH	6.39	6.39	6.39, 4.01, 3.00	6.39
temperature (°C)	30	30	30	30, 45, 60

[a] Groups G1–G4 are for studying the effects of ultrasonic power, initial solution concentration, initial solution pH, and temperature on the degradation of PYPS, respectively.

Purification of PYPS. A 300 mg amount of PYPS was put into a DEAE-52 column (2.6 cm × 60 cm) that had been equilibrated with the distilled water. After the column was washed with the distilled water, the absorbed materials were eluted with a gradient of NaCl (0.05, 0.1, 0.2, 0.3, and 0.5 mol/L) at a flow rate of 0.4 mL/min. The fractions containing total carbohydrate detected by the sulfuric acid−phenol method (*21*) and six fractions (PYPS1, PYPS2, PYPS3, PYPS4, PYPS5, and PYPS6) were collected.

Mensuration of Homogeneity and Molecular Weight of Fractions. PYPS1 to PYPS6 were mensurated by high performance gel permeation chromatography (HPGPC) fitted with GPC software on a column of Ultrahydrogel 1000, eluted by 0.001 mol/L NaOH. The column was precalibrated using standard T-Detrans (T-500, T-110, T-80, T-70, T-40, and T-9.3). All samples were prepared as 0.1% (w/v) solutions, and 20 μL of solution was injected in each run.

Ultrasonic Treatment. Ultrasonic irradiation was carried out using a HF-20B ultrasonic reactor (20 kHz, 100−1500 W, Beijing Hong Xiang Long Biotechnology Developing Co., Ltd.). The PYPS solution was placed in a reaction vessel, which was a 50 mL rosette cell, thermostated throughout the irradiation in a water bath (HH-S2, Chang Zhou Jin Tan Ltd.). To reduce the experimental errors caused by uneven power transfer, the sample was located just under the ultrasound source and dipped into sample about 20 mm. Irradiated samples were prepared in three replicates.

Effects of Ultrasonic Power, Initial Solution Concentration, Initial Solution pH, and Temperature on the Ultrasonic Degradation of PYPS. Effects on the ultrasonic degradation tested in the present work were arranged as follows (**Table 1**).

Analysis. The total carbohydrate content was measured by the sulfuric acid−phenol method using galactose as a standard (*22*). The protein content was measured by Lowry's method using bovine serum albumin as a standard (*23*). Crude fat was measured by the Soxhlet extraction procedure (*24*). PYPS's intrinsic viscosity [η], which is an index for indirectly appraising the degree of degradation, was measured by an Ubbelohde capillary (Ø 0.5−0.6 mm, 0.01187 mm^2/s^2) at 25 ± 0.1 °C. The [η] value was determined by the mean intercept of Huggins and Kraemer plots (*25*), using the Huggins equation

$$\frac{\eta_{sp}}{C} = [\eta] + k_H[\eta]^2 C \quad (1)$$

and the Kraemer equation

$$\frac{\ln\eta_{rel}}{C} = [\eta] - k_K[\eta]^2 C \quad (2)$$

where η_{rel}, η_{sp}, and [η] are the relative, specific, and intrinsic viscosities, respectively, and k_H and k_K are the Huggins and Kraemer constants, respectively.

RESULTS AND DISCUSSION

Isolation, Purification, Homogeneity, and Molecular Weight of PYPS. The investigated *P. yezoensis* contains 31.56% (w/w) of carbohydrates, 4.26% (w/w) of crude fat, and 44.16% (w/w) of protein on the basis of the starting dry weight. After extraction by supercritical CO_2, the crude fat in the residue is no more than 0.31% (w/w) of the residue dry weight. The protein content of the crude polysaccharides is decreased to 0.44% (w/w) after six times of Sevag's removal of the protein. The final yield of PYPS amounted to 6.24% (w/w) of the starting *P. yezoensis* dry weight and contained 94.59% (w/w) total carbohydrate of the fraction dry weight. Purification results of PYPS on DEAE-52 are shown in **Figure 1**. PYPS3, PYPS4, and PYPS5 showed a symmetrical peak in HPGPC, as shown in **Figure 2**. PYPS3, PYPS4, and PYPS5 molecular weights were determined to be 2.2 × 10^5, 3.8 × 10^5, and 6.1 × 10^5, respectively, in reference to standard dextrans.

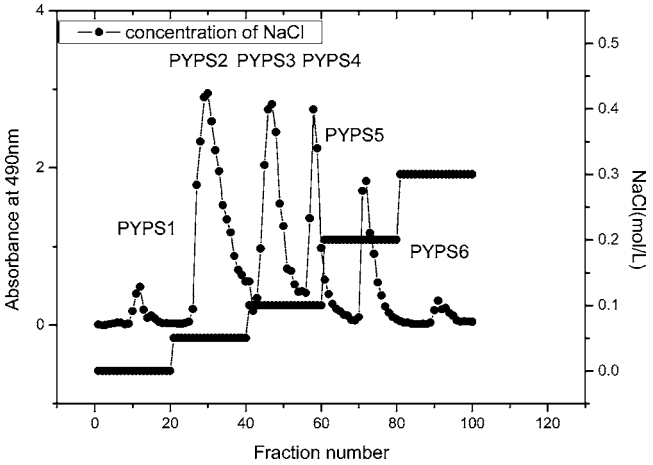

Figure 1. Elution profile of PYPS on a DEAE-52 column (2.6 cm × 60 cm).

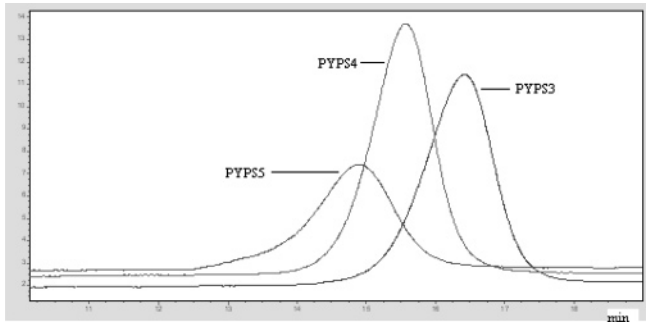

Figure 2. HPGPC chromatograms of PYPS3, PYPS4, and PYPS5.

Effect of Power on the Ultrasonic Degradation of PYPS. The effect of ultrasonic power on the ultrasonic degradation of PYPS solution (the conditions of experiment correspond to G1 in **Table 1**) is shown in **Figure 3**. It can be found that ultrasonic degradation of PYPS solution did not result in any foam formation. The intrinsic viscosity of PYPS decreases rapidly in the early 120 min of irradiation time and gradually tends to a constant value with time. The effect of the ultrasonic power on degradation of PYPS in the experimental time range from 0 to 240 min is that the intrinsic viscosity decreases with the ultrasonic power. Similar results can be found in ultrasonic degradation of dextrans, sodium dodecylbenzene sulfonate, and azo dye (*26*−*28*). Differentiating from lower frequency (20 kHz of this study), responsible effects of higher frequencies (>500

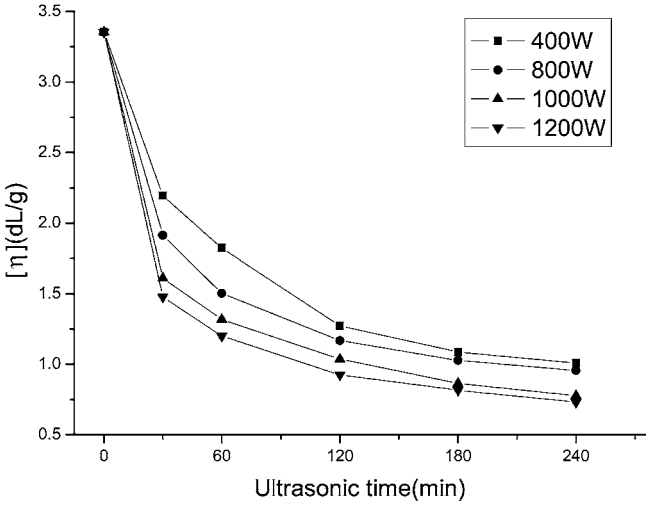

Figure 3. Ultrasonic degradation of PYPS at different powers.

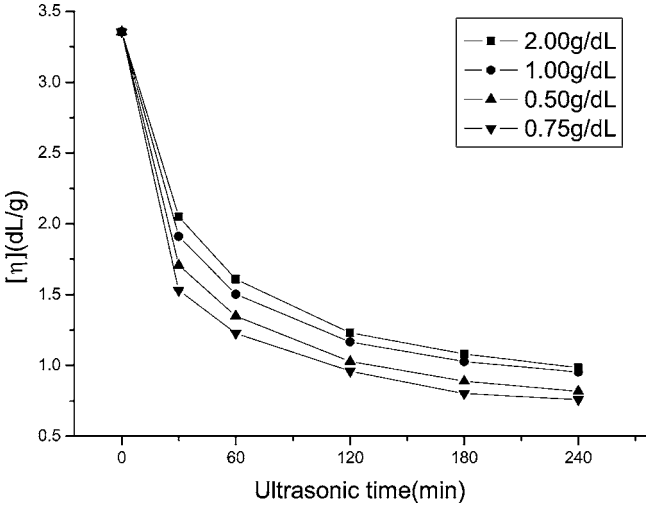

Figure 4. Ultrasonic degradation of PYPS at different concentrations.

kHz) on degradation are radical reactions (*29*). So, the effect of power on the sonolysis of PYPS may be explained by more by mechanical and less by radical effects. However, when the polysaccharide was degraded at the fixed ultrasonic power and frequency to a certain molecular weight, mechanical degradation cannot continue. As can be seen from **Figure 3**, the ultrasonic degradation curve corresponding to each ultrasonic power tends, respectively, to a constant [η] value. It indicates that there is a better corresponding relation between degradation extent and ultrasonic parameter.

Effect of Initial Solution Concentration on Ultrasonic Degradation of PYPS. The effect of initial solution concentration on ultrasonic degradation of PYPS solution in the concentration range of 0.50−2.00 g/dL at 30 °C, pH 6.39, and 800 W (corresponding to G2 in **Table 1**) is shown in **Figure 4**. The susceptibility of initial PYPS concentrations to degradation is at the order of 0.75 > 0.50 > 1.00 > 2.00 g/dL, just as Ashokkumar et al.'s conclusions (*30*), so 0.75 g/dL is the potential critical micelle concentration (CMC) of PYPS. This can also be confirmed by polysaccharide from *P. yezoensis* having an emulsifying ability as studied by Takahashi et al. (*31*). The same conclusion was also reported by Vinodgopal et al. (*32*).

Furthermore, the different polysaccharides from *P. yezoensis*, including the degraded one, basically have the same primary structure as follows: alternating (1→3)-linked β-D-galactose units and (1→4)-linked 3,6-anhydro-α-L-galactose units, which sometimes occur as the 6-sulfate and 2 or 6-*O*-methyl of galactose derivative (*33*, *34*). According to the PYPS's primary structure concluded and validated by Takahashi et al., it can be judged that PYPS is a kind of surfactant (*31*, *35*). The froth of surfactant can bring negative influence to ultrasonic wave transmit, further affecting the act of degradation. Consequently, decreasing the polysaccharides concentration to just above CMC's value could increase the process of ultrasonic degradation. If the CMC's value is increased up to a concentration of 5%, the reaction rate of ultrasonic degradation of trichloroethylene (TCE) decreased markedly. Micella sequestration of TCE seems to be the main reason for this additional inhibition (*36*).

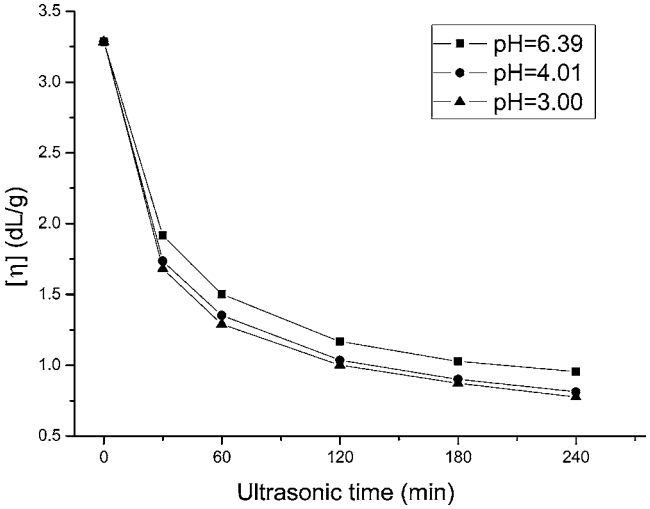

Figure 5. Ultrasonic degradation of PYPS at different pH values.

Effect of Initial Solution pH on the Ultrasonic Degradation of PYPS. Figure 5 shows the profiles on PYPS's initial solution pH irradiation time during ultrasonic degradation in the pH range of 6.39−3.00 at 30 °C, 1.00 g/dL, and 800 W (corresponding to G3 in **Table 1**). pH 6.39 is the pH value of the initial PYPS solution, and pH 4.01 and pH 3.00 were achieved by adding certain 0.5 mol/L amounts of HCl into the initial PYPS solution. The intrinsic viscosity of PYPS decreased with a decrease of the initial pH. In general, when HCl solutions are added, chemical reactivity is believed to be initiated by the acid hydrolysis. So, the partial reason of pH effect may be brought by acid hydrolysis. However, as compared to **Figure 5** with polysaccharides of acid hydrolysis, it can be found that ultrasonic degradation here is the main effect, and the process does not fully comply with a first-order reaction like acid hydrolysis (*37*, *38*). Furthermore, polysaccharides from *P. yezoensis* exhibited a high emulsifying activity index and a high emulsion stability over a wide range of pH values (3.0−8.0) (*31*), which can indirectly imply that the effect of initial solution pH on the ultrasonic degradation is not the background degradation and the real effect and mechanism should be further studied.

Effect of Temperature on the Ultrasonic Degradation of PYPS. The effect of irradiation time on the ultrasonic degradation of PYPS solution in the range of 30−60 °C for the 1.00 g/dL solution concentration at 800 W (corresponding to G4 in **Table 1**) is shown in **Figure 6**. The intrinsic viscosity of PYPS decreases rapidly in the first 2 h of irradiation time and gradually tends to a constant value with time. The effect of temperature on the ultrasonic degradation of PYPS in the experimental time range from 0 to 240 min is that the intrinsic viscosity decreases with the ultrasonic temperature.

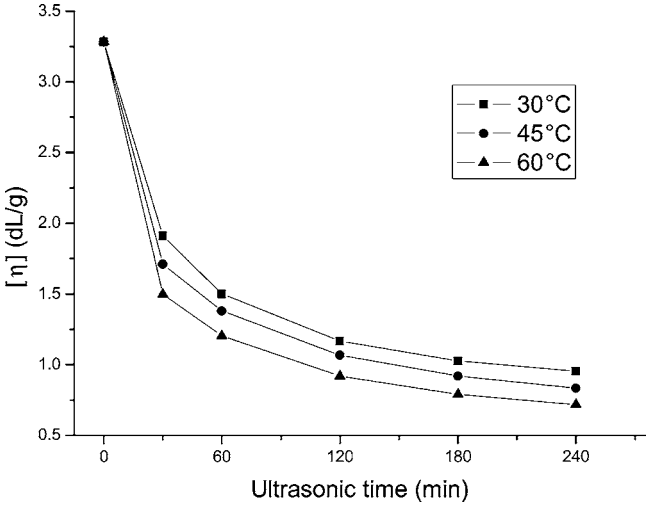

Figure 6. Ultrasonic degradation of PYPS at different temperatures.

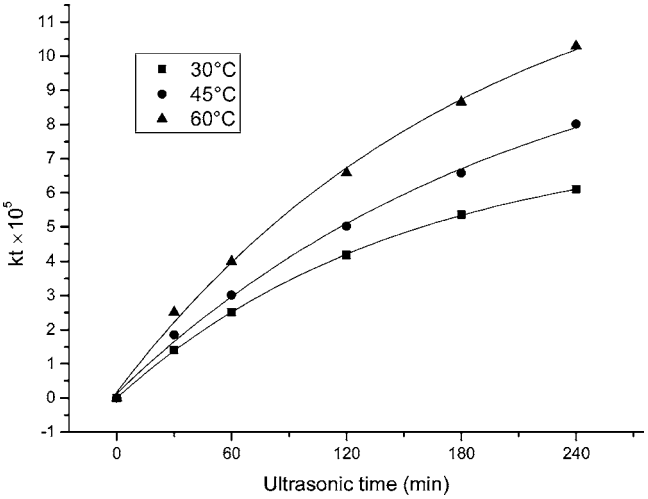

Figure 7. Overall rate expression for PYPS solution under ultrasonic degradation.

Calculation of the Activation Energy for Ultrasonic Degradation of PYPS. The activation energy is the minimum amount of energy required to initiate a reaction. It is one of the important indexes for appraising a reaction. The activation energy for ultrasonic degradation of PYPS is calculated as follows.

Like acid hydrolysis and thermal degradation of polysaccharide, the rate constant of the degradation can be obtained from the plot of the reciprocal molecular mass against time (eq 3) (*39*, *40*).

$$\frac{1}{M_t} - \frac{1}{M_0} = \frac{k}{m}t \tag{3}$$

where M_t and M_0 (kDa) are the molecular masses at time t and 0, respectively, k is the first-order rate constant, t (min) is ultrasonic time, and m (kDa) is the average molecular mass of a monosaccharide unit, based on the assumption that α(1→3) and β(1→4) glycosidic linkages in these polysaccharides show similar susceptibility to breaks by ultrasound.

The combination of eq 3 and Mark−Houwink eq 4.1:

$$[\eta] = K_{\mathrm{MH}} \times M^{\alpha} \tag{4.1}$$

gives theoretically a relationship between $[\eta]$ and t as follows:

$$\frac{1}{[\eta]_t^{1/\alpha}} - \frac{1}{[\eta]_0^{1/\alpha}} = \frac{kt}{m \times K_{\mathrm{MH}}{}^{1/\alpha}} \tag{5}$$

where $[\eta]_t$ and $[\eta]_0$ (mL/g) are the intrinsic viscosities at time t and 0, respectively; K_{MH} and α are constants for a given system, which can be found from the logarithmic form of Mark−Houwink eq 4.2:

$$\log [\eta] = \log K_{\mathrm{MH}} + \alpha \log M_{\eta} \tag{4.2}$$

where $[\eta]$ (mL/g) is intrinsic viscosity and M_{η} is the average viscosity molecular weight.

For the system of this study, $K_{\mathrm{MH}} = 0.00786$ and $\alpha = 0.626$ of PYPS at 25 °C was found by using PYPS3, PYPS4, and PYPS5. The $m = 0.18$ kDa for PYPS was obtained from the literature (*41*).

The kt dependence of the degradation time for ultrasonic degradation of PYPS solution at temperatures of 30, 45, and 60 °C is shown in **Figure 7** and illustrates that for the cases an exponential curve exists between kt of the PYPS solution and the degradation time. From this dependence, k was evaluated by exponential regression analysis (eqs 6−8) of the data points (**Figure 7**), first derivative (eqs 9−11), and limit (eqs 12−14).

$$kt \times 10^5 = -7.679e^{(-t/151.690)} + 7.686\ (R^2 = 0.99996),\ 30\ ^{\circ}\mathrm{C} \tag{6}$$

$$kt \times 10^5 = -11.451e^{(-t/181.762)} + 11.590\ (R^2 = 0.99800),\ 45\ ^{\circ}\mathrm{C} \tag{7}$$

$$kt \times 10^5 = -13.912e^{(-t/188.523)} + 14.092\ (R^2 = 0.99798),\ 60\ ^{\circ}\mathrm{C} \tag{8}$$

$$k \times 10^5 = 0.051e^{(-t/151.690)},\ 30\ ^{\circ}\mathrm{C} \tag{9}$$

$$k \times 10^5 = 0.063e^{(-t/211.321)},\ 45\ ^{\circ}\mathrm{C} \tag{10}$$

$$k \times 10^5 = 0.074e^{(-t/188.523)},\ 60\ ^{\circ}\mathrm{C} \tag{11}$$

$$\lim_{t\to 0} 0.051e^{(-t/151.690)} = 0.051,\ 30\ ^{\circ}\mathrm{C} \tag{12}$$

$$\lim_{t\to 0} 0.063e^{(-t/211.321)} = 0.063,\ 45\ ^{\circ}\mathrm{C} \tag{13}$$

$$\lim_{t\to 0} 0.074e^{(-t/188.523)} = 0.074,\ 60\ ^{\circ}\mathrm{C} \tag{14}$$

The temperature dependence of k for PYPS solution is given in **Figure 8**. An Arrhenius rate law type (eq 15) dependent on ln(k) against T^{-1} is observed in **Figure 8**. From this dependence, the activation energy (E_a) was evaluated by linear regression analysis of the data points. The activation energy for ultrasonic degradation of PYPS solution is 52.13 kJ/mol, which was evaluated from the logarithmic form of the Arrhenius eq 20 by plotting ln(k) against T^{-1}:

$$\ln k = -\frac{E_a}{RT} + \ln A \tag{15}$$

where A is the Arrhenius constant, R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature.

Comparing carrageenan (κ- and ι-) with PYPS having a similar backbone structure of α(1→3) and β(1→4) glycosidic of galactose, its activation energy of acid hydrolysis is from 120 to 190 kJ/mol (*42*). The activation energy of β-galactosidase

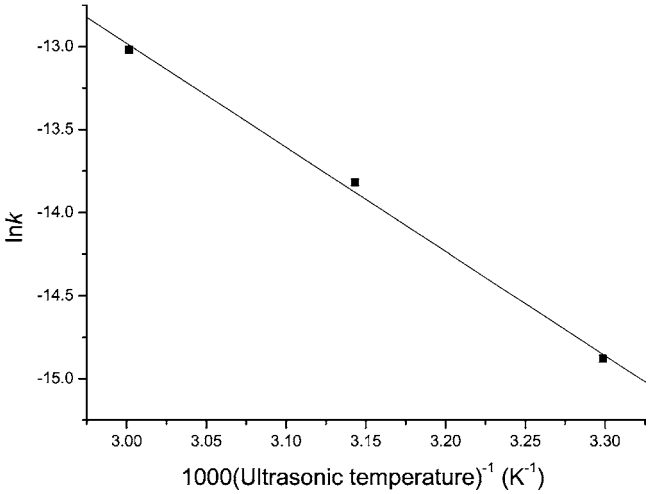

Figure 8. Temperature dependence of the *k* for polysaccharide solution under ultrasonic degradation.

(EC 3.2.1.23) at optimum temperature (65 °C) is about 100.8 kJ/mol (*43*). The activation energy of α-galactosidase at maximal activity is 68.88 kJ/mol (*44*). Therefore, as activation energy of ultrasonic degradation of PYPS to be concert, ultrasound could be a potential and useful way to obtain desired molecular weights of polysaccharides. Furthermore, controlling the degradation of PYPS is useful in order to control properties such as viscosity, solubility, and ever biological activity. Thus, establishing relationships between molecular weights and properties (viscosity, solubility, biological activity, and so on) and even the corresponding ultrasonic degradation conditions (ultrasonic power, frequency, temperature, pH, and so on) are interesting, necessary, and of importance.

In conclusion, the changes of intrinsic viscosity under various conditions, such as ultrasonic power, initial concentration, initial pH of solution, and reaction temperature, were investigated by Ubbleholde viscometry. It was found that the ultrasonic degradation rate of PYPS solution increases with the increase of ultrasonic power and reaction temperature and the decrease of initial solution pH. The order of initial PYPS concentrations susceptibility to degradation is 0.75 > 0.5 > 1.00 > 2.00 g/dL. The mechanism in the ultrasonic degradation of PYPS may be explained more by mechanical and less by radical effects. Relationships between degradation rate and ultrasound time are exponential functions. The activation energy of ultrasonic degradation of PYPS solution is 52.13 kJ/mol, which was calculated by the logarithmic form of the Arrhenius equation. The activation energy for the degradation is lower than one for the acid degradation and for the enzyme catalyzing degradation of similar glycosidic bonds.

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LITERATURE CITED

(1) Gallant, D.; Degrois, M.; Sterling, C.; Guilbot, A. Microscopic effects of ultrasound on the structure of potato starch. *Die Starke* **1972**, *24*, 116−123.

(2) Mendez, J.; Franklin, B.; Kollias, J. Effect of ultrasound on the permeability of D_2O through cellulose membranes. *Biomedicine* **1976**, *25*, 121−122.

(3) Aliyu, M.; Hepher, M. J. Effects of ultrasound energy on degradation of cellulose material. *Ultrason. Sonochem.* **2000**, *7*, 265−268.

(4) Aimin, T.; Hongwei, Z.; Gang, C.; Guohui, X.; Wenzhi, L. Influence of ultrasound treatment on accessibility and regioselective oxidation reactivity of cellulose. *Ultrason. Sonochem.* **2005**, *12*, 467−472.

(5) Lockwood, A. R.; James, A. E.; Pautard, F. G. Studies on the breakdown product of dextran formed by ultrasonic vibration. *Research* **1951**, *4*, 46−48.

(6) Stacey, M. Degradation of dextran by ultrasonic waves. *Research* **1951**, *4*, 48.

(7) Glaeser, W.; Berg, H. Physicochemical studies on dextran. VI. The sedimentation and diffusion behavior of dextran after ultrasonic treatment. *Pharmazie* **1959**, *14*, 380−392.

(8) Zorina, O. M.; Ie, E. Physico-chemical changes in dextran (polyglucin) caused by ultrasonic waves. *Biokhimiia* **1963**, *28*, 781−785.

(9) Cravotto, G.; Tagliapietra, S.; Robaldo, B.; Trotta, M. Chemical modification of chitosan under high-intensity ultrasound. *Ultrason. Sonochem.* **2005**, *12*, 95−98.

(10) Yoshizawa, Y.; Ametani, A.; Tsunehiro, J.; Nomura, K.; Itoh, M.; Fukui, F.; Kaminogawa, S. Macrophage stimulation activity of the polysaccharide fraction from a marine alga (Porphyra yezoensis): Structure−function relationships and improved solubility. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1933−1937.

(11) Yoshizawa, Y.; Enomoto, A.; Todoh, H.; Ametani, A.; Kaminogawa, S. Activation of murine macrophages by polysaccharide fractions from marine algae (Porphyra yezoensis). *Biosci., Biotechnol., Biochem.* **1993**, *57*, 1862−1866.

(12) Inui, H.; Tsujikubo, M.; Hirano, S. Low molecular weight chitosan stimulation of mitogenic response to platelet-derived growth factor in vascular smooth muscle cells. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 2111−2114.

(13) Kim, T. H.; Ihm, J. E.; Choi, Y. J.; Nah, J. W.; Cho, C. S. Efficient gene delivery by urocanic acid-modified chitosan. *J. Controlled Release* **2003**, *93*, 389−402.

(14) Renata, C. B.; Bozena, B.; Salah, L.; Pitor, U.; Janusz, M. R. Degradation of chitosan and starch by 360-kHz ultrasound. *Carbohydr. Polym.* **2005**, *60*, 175−184.

(15) Fonty, G.; Williams, A. G.; Bonnemoy, F.; Withers, S. E.; Gouet, P. Effect of anaerobic fungi on glycoside hydrolase and polysaccharide depolymerase activities, in sacco straw degradation and volatile fatty acid concentrations in the rumen of gnotobiotically reared lambs. *Reprod., Nutr., Dev.* **1995**, *35*, 329−337.

(16) Dawson, J. E.; Hajratwala, B. R.; Taylor, H. Kinetics of indomethacin degradation II: Presence of alkali plus surfactant. *J. Pharm. Sci.* **1977**, *66*, 1259−1263.

(17) Jacobs, W. A.; Craig, L. C. The ergot alkaloids. II. The degradation of ergotinine with alkali. *J. Biol. Chem.* **2002**, *277*, 26−30.

(18) Berry, S. M.; Hong, B. S.; Lam, K. W.; Haddox, J. L.; Pfister, R. R. Degradation of bovine corneal collagen by alkali. *Cornea* **1989**, *8*, 150−154.

(19) Zhang, W. Y.; Li, Y.; Chen, H.; Tan, R. X.; Hou, Y. Y. Preparation of polysaccharide from *Porphyra yezoensis* Ueda and its effects on growth of lymphocytes. *J. Wuhan Bot. Res.* **2001**, *19*, 149−152.

(20) Wirdez, R. L. Analysis and preparation of sugars. *Methods in Carbohydrate Chemistry*; Academic Press: New York and London, 1965; Vol. 1, p 23.

(21) Chen, Y. C.; Vorontsov, A. V.; Smirniotis, P. G. Enhanced photocatalytic degradation of dimethyl methylphosphonate in the presence of low-frequency ultrasound. *Photochem. Photobiol. Sci.* **2003**, *2*, 694−698.

(22) Kellen, J.; Vajkova, M. Determination of blood sugar with phenol-sulfuric acid. *Z. Gesamte Inn Med.* **1961**, *16*, 188−196.

(23) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

(24) Sporring, S.; Bowadt, S.; Svensmark, B.; Bjorklund, E. Comprehensive comparison of classic Soxhlet extraction with Soxtec extraction, ultrasonication extraction, supercritical fluid extraction, microwave assisted extraction and accelerated solvent extraction for the determination of polychlorinated biphenyls in soil. *J. Chromatogr. A* **2005**, *1090*, 1−9.
(25) Young, R. J.; Pirt, A. L. *Introduction to Polymers*, 2nd ed.; Chapman: New York, 1991; pp 138−166.
(26) Lorimer, J. P.; Cuthbert, T. C.; Brookfield, E. A. Effect of ultrasound on the degradation of aqueous native dextran. *Ultrason. Sonochem*. **1995**, *2*, 55−57.
(27) Manousaki, E.; Psillakis, E.; Kalogerakis, N.; Mantzavinos, D. Degradation of sodium dodecylbenzene sulfonate in water by ultrasonic irradiation. *Water Res*. **2004**, *38*, 3751−3759.
(28) Astrid, R.; Michael, T.; Georg, G. Application of power ultrasound for azo degradation. *Ultrason. Sonochem*. **2004**, *11*, 177−182.
(29) Portenlanger, G.; Heusinger, H. The influence of frequency on the mechanical and radical effects for the ultrasonic degradation of dextranes. *Ultrason. Sonochem*. **1997**, *4*, 127−130.
(30) Ashokkumar, M.; Niblett, T.; Tantiongco, L.; Grieser, F. Sonochemical degradation of sodium dodecylbenzene sulfonate in aqueous solutions. *Aust. J. Chem.* **2003**, *56*, 1045−1049.
(31) Takahashi, K.; Hirano, Y.; Araki, S.; Hattori, M. Emulsifying ability of porphyran prepared from dried nori, Porphyra yezoensis, a red alga. *J. Agric. Food Chem*. **2000**, *48*, 2721−2725.
(32) Vinodgopal, K.; Ashokkumar, M.; Grieser, F. Sonochemical degradation of a polydisperse nonylphenol ethoxylate in aqueous solution. *J. Phys. Chem. B* **2001**, *105*, 3338−3342.
(33) Zhang, W. Y.; Chen, H.; Wang, S. J.; Tan, R. X.; Fei, X. G. The isolation, pufification and Identification of a kind of agar polysaccharide from *Porphyra yezoensis*. *Chin. Bull. Bot.* **2000**, *17*, 429−434.
(34) Morric, L. M.; Maclean, M. W.; Long, W. F.; Williamson, F. B. Porphyran primary structure. *Eur. J. Biochem*. **1983**, *133*, 676−684.
(35) Hirano, Y.; Hattori, M.; Takahashi, K. Interaction of porphyran with a hydrophobic surface and stabilization of liposomes. *J. Agric. Food Chem.* **2005**, *53*, 9800−9804.
(36) Destaillats, H.; Alderson, T. W., 2nd; Hoffmann, M. R. Applications of ultrasound in NAPL remediation: Sonochemical degradation of TCE in aqueous surfactant solutions. *Environ. Sci. Technol.* **2001**, *35*, 3019−3024.
(37) Hjerde, T.; Smidsrød, O.; Christensen, B. E. The influence of the conformational state of *k*- and *l*- carrageenans: Characterization of partially hydrolysis. *Carbohydr. Res*. **1996**, *288*, 175−187.
(38) Hjerde, T.; Stokke, B. T.; Christensen, B. T. Acid hydrolysis of *k*- and *l*-carrageenans: Characterization of partially hydrolyzed samples and single-stranded oligomers released from the orderd structure. *Macromolecules* **1998**, *31*, 1842−1851.
(39) Myslabodski, D. E.; Stancioff, D.; Heckert, R. A. Effect of acid hydrolysis on the molecular weight of kappa carrageenan by GPC-LS. *Carbohydr. Polym*. **1996**, *31*, 83−92.
(40) Singh, S. K.; Jacobsson, S. P. Kinetics of acid hydrolysis of carrageen as determined by molecular weight (SEC-MALLS-R), gel breaking strength, and viscosity measurements. *Carbohydr. Polym*. **1994**, *23*, 89−103.
(41) Cyrille, R.; Marc, L. Average molecular weight and molecular weight distribution of agarose and agarose-type polysaccharides. *Carbohydr. Polym*. **1989**, *10*, 289−298.
(42) Torgeir, H.; Olav, S.; Bjørn, E. Christensen. The influence of the conformational state of κ- and λ-carrageenan on the rate of acid hydrolysis. *Carbohydr. Res.* **1996**, *288*, 175−187.
(43) Goodman, R. E.; Pederson, D. M. beta-Galactosidase from Bacillus stearothermophilus. *Can. J. Microbiol.* **1976**, *22*, 817−825.
(44) Lusis, A. J.; Paigen, K. Properties of mouse alpha-galactosidase. *Biochim. Biophys. Acta* **1976**, *437*, 487−497.

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