

**Degradation and Isomerization of Monosaccharides and
Their Derivatives in Subcritical Water**

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2017

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General Introduction

Figure I shows the schematic phase diagram of Water. When sufficient pressure is applied, water can exist as a liquid even at temperatures above its boiling point. Water that maintains its liquid state under pressurized conditions in the temperature range of 100°C to 374°C is called subcritical water (shaded region in Fig. I).

Figure II shows the change in the relative dielectric constant, ϵ , and ion product, K_w , of water as a function of temperature at 10 MPa. The relative dielectric constant decreases 79.0 at 25°C to 35.3 at 200°C and 20.7 at 300°C which are comparable to those of methanol and acetone under ambient conditions (Yang *et al.*, 1998; Eckert *et al.*, 2000). Due to this property, water can be used to extract hydrophobic substances from various resources (Rodríguez-Meizoso *et al.*, 2006; Hashimoto *et al.*, 2004; Santos *et al.*, 2005). As the temperature of water increases from boiling point to critical point, the ion product, K_w , of water increases with temperature by more than three orders of magnitude from the value of $10^{-14}(\text{mol/L})^2$ at 25°C to the value of 10^{-12} to $10^{-11}(\text{mol/L})^2$ at the temperature range of 150 to 250°C. Because this fact means that the concentrations of hydrogen and hydroxyl ions are

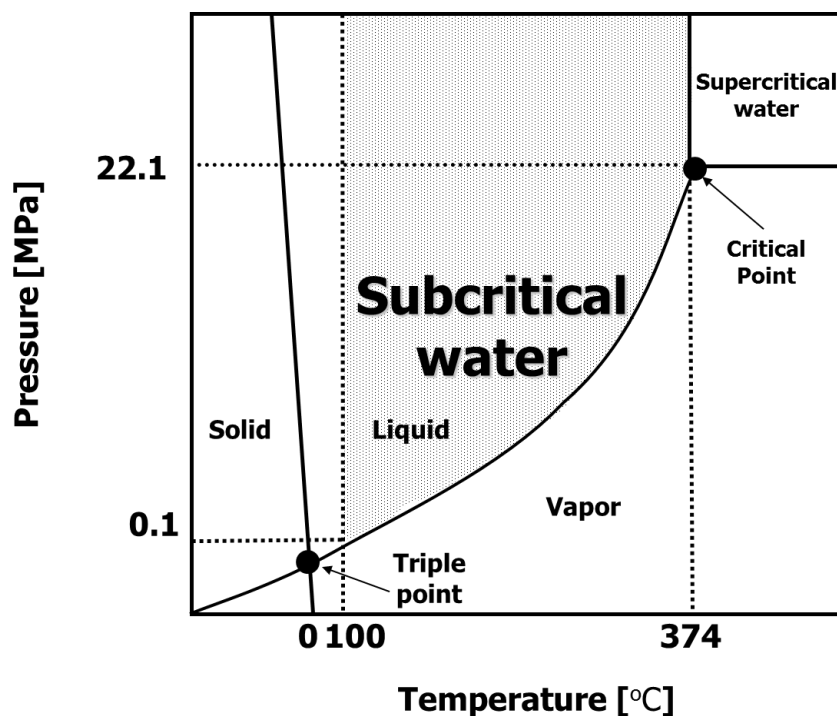


Figure I. Schematic phase diagram of water

high, there is the possibility that the water can act as an acid or base catalyst without the addition of any catalyst (Kramer and Vogel, 2000; Kuhlmann *et al.*, 1994; Lesutis *et al.*, 1999). Based on this feature, the hydrolysis of vegetable oil (Holliday *et al.*, 1997) and degradation of polycyclic hydrocarbons (Yang and Hilderbrand, 2006), condensation (Sasaki *et al.*, 2002) and isomerization (Kabyemela *et al.*, 1997) by subcritical water have been reported.

The most important advantages of using subcritical water as a solvent or reaction medium are relatively low cost, safety, and replacement of environmentally undesirable acid catalysts. Many reactions can occur in subcritical water without the addition of acid or base catalyst because protons and hydroxyl ions resulted from the high ion product of water would catalyze the reactions under subcritical conditions. Therefore, various reactions using subcritical water is utilized as the new technology for biomass processing, waste treatment, and material synthesis.

The aim of this study is obtained basic knowledge of the phenomena that occur during reactions in subcritical water. The author studied kinetically for degradation and isomerization of monosaccharides, their derivatives, and fatty acid in the subcritical water in five chapters.

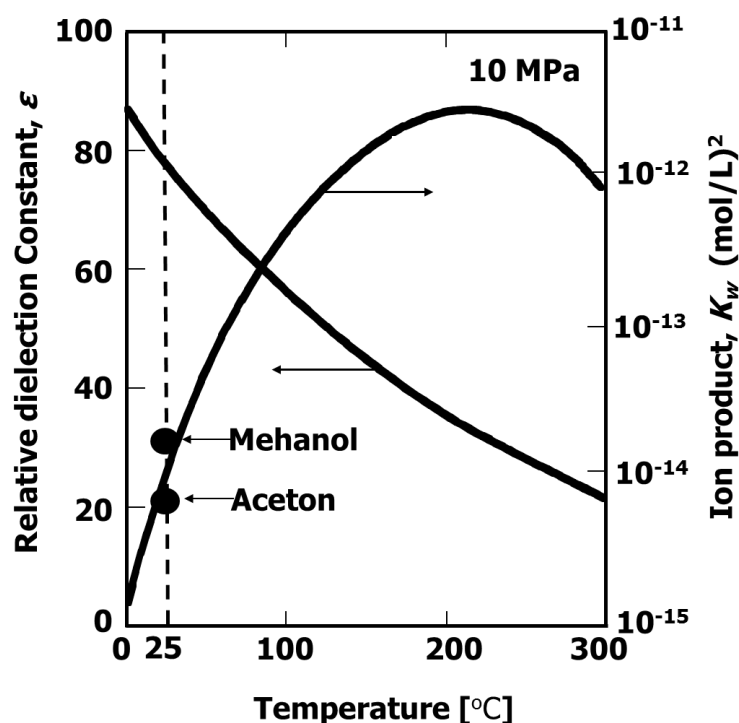


Figure II. Schematic phase diagram and change in the relative dielectric constant and ion product of water with temperature at 10 MPa.

In chapter 1, the decomposition of four pentoses (xylose, arabinose, lyxose, and ribose) and two hexouronic acids (glucronic acid and galactronic acid) in the subcritical water are measured, and the decomposition processes are kinetically analyzed.

In chapter 2, the isomerizations of mannose, fructose, and glucose in subcritical water are examined, and their kinetics are discussed to investigate the catalytic properties of subcritical water.

In chapter 3, it was determined whether the isomerization of linoleic acid to its conjugated isomers occurs in subcritical water or not, and what type of isomer is apt to be formed if the isomerization occurs.

In chapter 4 and 5, the effects of adding ethanol in subcritical water for the disappearance of *N*-acetyl-D-glucosamine (GlcNAc), and interconversion between D-glucuronic acid (GlcA) and D-glucuronolactone (GlcL), respectively, are investigated. In chapter 4, the disappearance of GlcNAc in subcritical aqueous ethanol is examined and kinetically analyzed. In chapter 5, the interconversion between GlcA and GlcL in subcritical aqueous ethanol is examined and kinetically analyzed.

Chapter 1

Degradation of Pentaoses and Hexouronic Acids in Subcritical Water

1.1 Introduction

Effective utilization of the biomass through its hydrolysis or degradation by the sub- or supercritical water has also been reported (Schmieder and Abelin, 1999; Yoshida *et al.*, 2004; Mok and Antal Jr., 1992; Miyazawa and Funazukuri, 2004; Liu and Wyman, 2005). Hemicellulose is a major component of biomass, and consists of pentoses, hexoses, and hexouronic acids. It has been reported that the monosaccharides produced through the hydrolysis of hemicellulose by the subcritical water were further degraded and that the degradation resulted in the lowering of the overall yield of the monosaccharides (Heitz *et al.*, 1991; Abatzoglou *et al.*, 1992; Saddler *et al.*, 1993). However, the decomposition kinetics of the constituents of the hemicellulose by the subcritical water seems to have not been analyzed in detail, although we have reported the degradation kinetics of hexoses (Haghighat Khajavi *et al.*, 2005).

In this context, we measured the decomposition of four pentoses (xylose, arabinose, lyxose, and ribose) and two hexouronic acids (glucuronic acid and galacturonic acid) in the subcritical water, and the decomposition processes were kinetically analyzed. Figure 1-1 shows the structural formulas of the substrates.

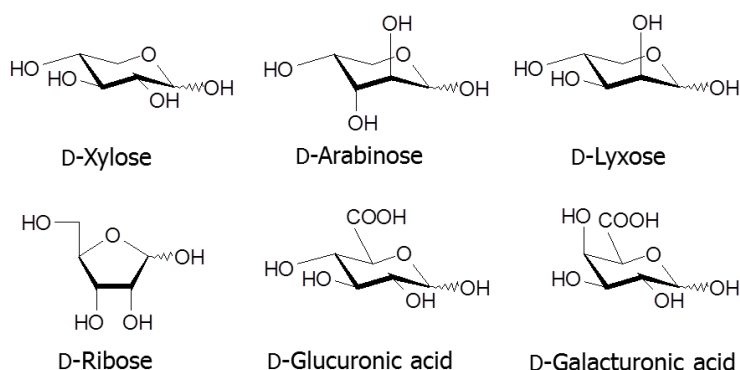


Figure 1-1. Structural formulas of the pentaoses and hexauronic acids used as the substrates

1.2 Materials and Methods

1.2.1 Materials

D-(-)-Arabinose, D-(+)-xylose, D-(+)-galacturonic acid (monohydrate), and D-glucuronic acid (purity > 98%) were purchased from Wako Pure Chemical Industries, Osaka, Japan. D-Ribose (> 98%) and D-lyxose were purchased from Sigma-Aldrich Japan, Tokyo, Japan. Frufural was obtained from Nacalai Tesque, Kyoto, Japan.

1.2.2 Degradation of pentose or hexouronic acid

Figure 1-2 schematically shows the flow-type apparatus used for the degradation of the pentoses and hexouronic acids in the subcritical water. A pentose or hexouronic acid was dissolved in distilled water at 0.5% (w/v), which corresponds to 33.3 mmol/L and 25.8 mmol/L for the pentose and hexouronic acid, respectively. After the solution was sonically degassed, a helium gasbag was connected to the solution bottle to prevent the dissolution of oxygen. The substrate solution was fed at a constant flow rate by an LC-10AT pump (Shimadzu, Kyoto) to the tubular reactor, which was a stainless steel (SUS316) coil of 0.8 mm I.D. and 0.5 or 3 m length and was immersed in a bath filled with a SRX310 silicone oil (Toray-Dow Corning Silicone, Tokyo). The mean residence time in the reactor was calculated from the flow rate of the feed solution at room temperature and the densities of the water at the room and experimental temperatures. The solution leaving the reactor passed through a

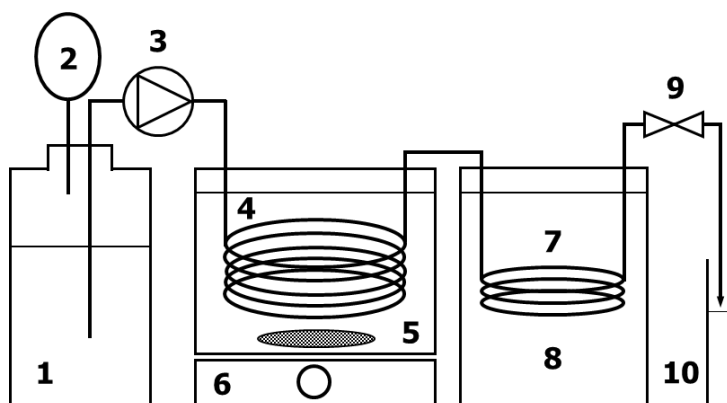


Figure 1-2. Schematic diagram of the experimental apparatus. 1:reservoir of feed solution, 2:helium gasbag, 3:pump, 4:coiled stainless steel tube (tubular reactor), 5:oil bath, 6:stirrer, 7:cooling coil, 8:iced water bath, 9:back-pressure valve, and 10:sampling tube.

coil (0.8 mm I.D., 1.0 m length) immersed in iced-water to stop the reaction. It was roughly estimated from the properties of water and thermal conductivity of stainless steel that the temperature reached 98% of a desired level within a few seconds at the reactor inlet in every case. The estimation is also applicable to the temperature at the reactor outlet. Because the time was much shorter than the residence time, the temperature changes at the inlet and outlet could be approximated to be stepwise. The system pressure was maintained at 10 MPa using a high pressure adjustable back-pressure valve (Upchurch Scientific, Oak Harbor, WA, USA). After ten times longer than the residence time in the reactor had elapsed, the effluent from the reactor was sampled to measure the remaining substrate, the produced furfural and pH.

1.2.3 Analysis

The pentose or hexouranic acid remaining in the effluent was determined using a Shimadzu LC-6A HPLC equipped with a Supelcogel Ca column (7.8 mm I.D. $\times$ 30 cm, Supelco, Bellefonte, PA, USA), which was held at 80°C in an oven, and an RID-6A refractometer (Shimadzu). The eluent was distilled water flowing at 0.5 mL/min.

Furfural was also determined by HPLC consisting of an LC-10AD pump (Shimadzu), an ODS-A column (4.6 mm I.D. $\times$ 10 cm, YMC, Kyoto) and an SPD-10Avp UV detector (Shimadzu, 284 nm). The eluent was a methanol-water mixture (10:90 by vol.) at 0.5 mL/min. The measurement was carried out at room temperature.

The pH of the effluent was measured at room temperature using an F-13 pH meter (Horiba, Kyoto).

1.3 Results and Discussion

1.3.1 Degradation of pentoses

Figure 1-3 shows the fractions of the remaining substrate, produced furfural and pH of the reactor effluent at various residence times for the degradation of xylose at 200, 220, and 240°C. Xylose and furfural were more rapidly degraded and formed, respectively, at the higher temperature. The pH of the reactor effluent steeply decreased at the short residence time and leveled off at *ca.* 3 at any temperature. The degradation of arabinose, ribose, and

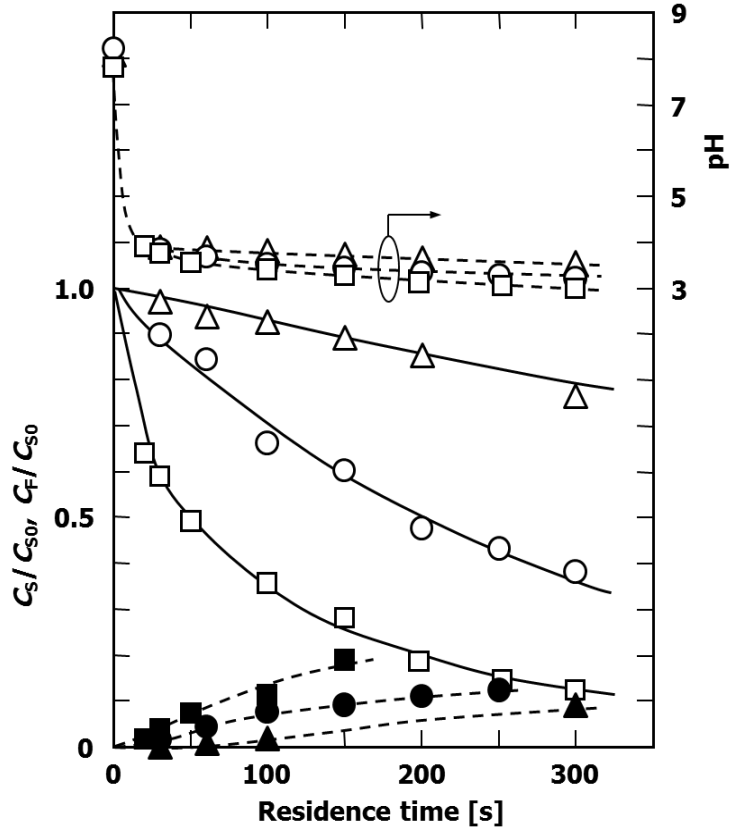


Figure 1-3. Degradation of (open symbols) xylose in subcritical water at ($\triangle$, $\blacktriangle$) 200°C, ($\circ$, $\bullet$) 220°C, and ($\square$, $\blacksquare$) 240°C, and (closed symbols) formation of furfural and (open symbols) pH of the reactor effluent during the degradation. C_{S0} is the xylose concentration in the feed solution, and C_S and C_F are the concentrations of the remaining xylose and formed furfural, respectively, in the reactor effluent.

lyxose was also measured at the same temperatures as that of xylose. Figure 1-4 shows the degradation of the pentoses at 220°C. Ribose was the most rapidly degraded, and arabinose was the most resistant to the degradation among the tested pentoses. Furfural was more rapidly produced from the pentose which was more rapidly degraded. The pH change with the residence time was almost the same for all the pentoses, and the value rapidly decreased to *ca.* 3.

Because the change in the fraction of the remaining substrate with the residence time did not obey the simple first-order kinetics, the Weibull model of Eq. (1-1) (Cunha *et al.*, 1998) was adopted to describe the change for all the pentoses at any temperature:

$$C_S / C_{S0} = \exp[-(k\tau)^n] \quad (1-1)$$

where C_S and C_{S0} are the concentrations of a substrate in the reactor effluent and in the feed

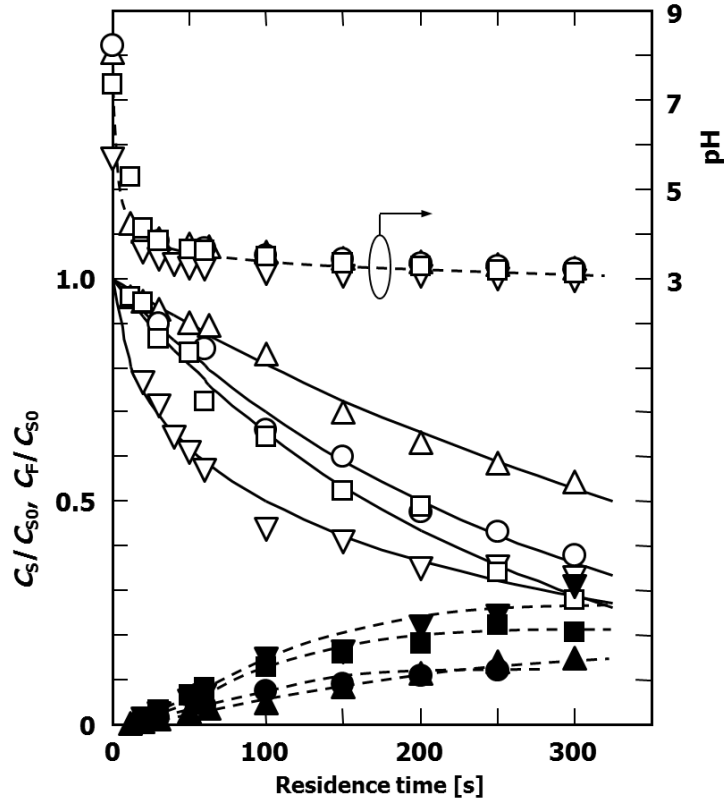


Figure 1-4. Changes in (open symbols) the fraction of remaining substrate, (closed symbols) the fraction of produced furfural and (open symbols) pH of the reactor effluent during the degradation of ($\triangle$, $\blacktriangle$) arabinose, ($\circ$, $\bullet$) xylose, (∇ , $\blacktriangledown$) ribose, and ($\square$, $\blacksquare$) lyxose in subcritical water at 220°C.

reservoir, respectively, τ is the mean residence time of the substrate solution in the reactor, k is the rate constant, the reverse of which is called the scale constant, and n is the shape constant. The Weibul model, which is also called the Avrami model in the field of crystallization, has been successfully applied to describe many processes such as degradation, deterioration and drying (Cunha *et al.*, 1998). The kinetic parameters, k and n , were evaluated by fitting the experimental results by a nonlinear regression using the Solver of Microsoft Excel[®] for Windows[®] (Harris, 1998; Levie 1999). The solid curves in Figs. 1-3 and 1-4 were drawn by substituting the estimated parameters in Eq. (1-1).

1.3.2 Degradation of hexouronic acids

Since the hexouronic acids were more susceptible to degradation than the pentoses, their degradation was observed at temperatures lower than the pentoses. Figure 1-5 shows the degradation processes of the glucuronic and galacturonic acids at 140, 150, and 160°C.

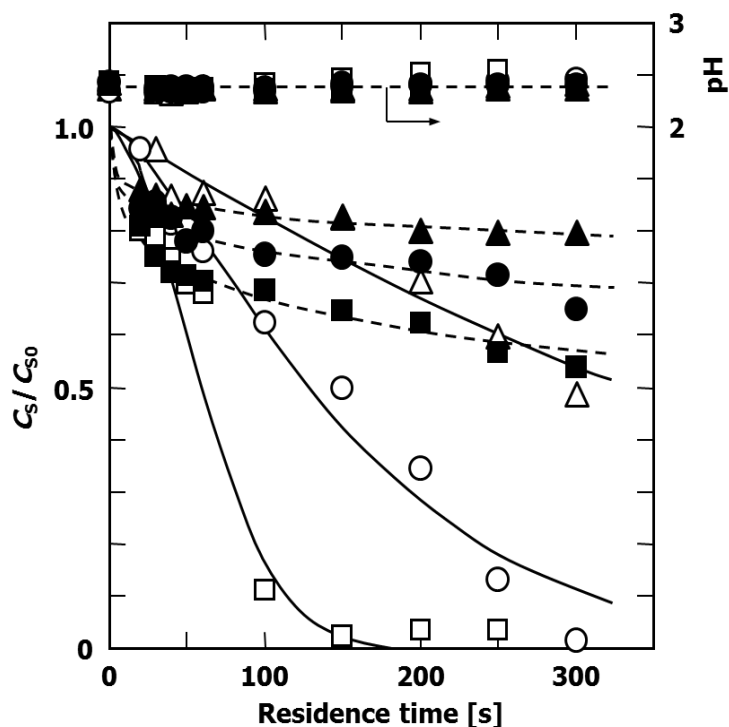


Figure 1-5. Degradation of (open symbols) glucuronic acid and (closed symbols) galacturonic acid in subcritical water at ($\triangle$, $\blacktriangle$) 140°C, ($\circ$, $\bullet$) 150°C, and ($\square$, $\blacksquare$) 160°C, and pH of the reactor effluent during the degradation.

Furfural was scarcely produced from the hexouronic acids. Therefore, their concentrations are not shown in the figure although they were determined. Glucuronic acid was more rapidly degraded at the higher temperature, and it completely disappeared after a prolonged residence time. The degradation behavior of the galacturonic acid was quite different from that of the glucuronic acid. Galacturonic acid was rapidly degraded to a certain level at the short residence time, and then it very slowly degraded.

The degradation processes of both the glucuronic and galacturonic acids were expressed by the Weibull model. The kinetic parameters were evaluated by a previously described method. The solid and broken curves in Fig. 1-5 were drawn using the estimated parameters for the glucuronic and galacturonic acids, respectively.

Because the glucuronic and galacturonic acids are acidic compounds, their solutions exhibited low pH values of *ca.* 2.4. No significant change in the pH value was observed during the degradation of both the glucuronic and galacturonic acids, as shown in Fig. 1-5.

1.3.3 Temperature dependences of kinetic parameters

As shown in Figs. 1-6-a1 and 1-6-b1, the temperature dependence of the rate constant k could be expressed by the Arrhenius equation for the degradation of all the substrates:

$$k = k_0 \exp(-E/RT) \quad (1-2)$$

where E is the activation energy, k_0 is the frequency factor, R is the gas constant, and T is the absolute temperature. The E and k_0 values for each substrate were evaluated from the plots shown in Fig. 1-6-a1 or 1-6-b1. The E values were in the range of 100 to 150 kJ/mol except for galacturonic acid, the E value of which was 427 kJ/mol. Figures 1-6-a2 and 1-6-b2 show the temperature dependences of the n value for the pentoses and hexouronic acids, respectively. The n values for the pentoses were in a range from 0.7 to 1.3. The reason why the degradation could not be expressed by the first-order kinetics remains unclear. A possible reason for the degradation of the pentoses was a rapid decrease in the pH for the short residence times. However, the n values for the degradation of the hexouronic acids were not unity although the pH did not significantly change during the degradation. The low n values for the galacturonic acid reflected the rapid decrease in the fraction of the remaining substrate at the short residence times.

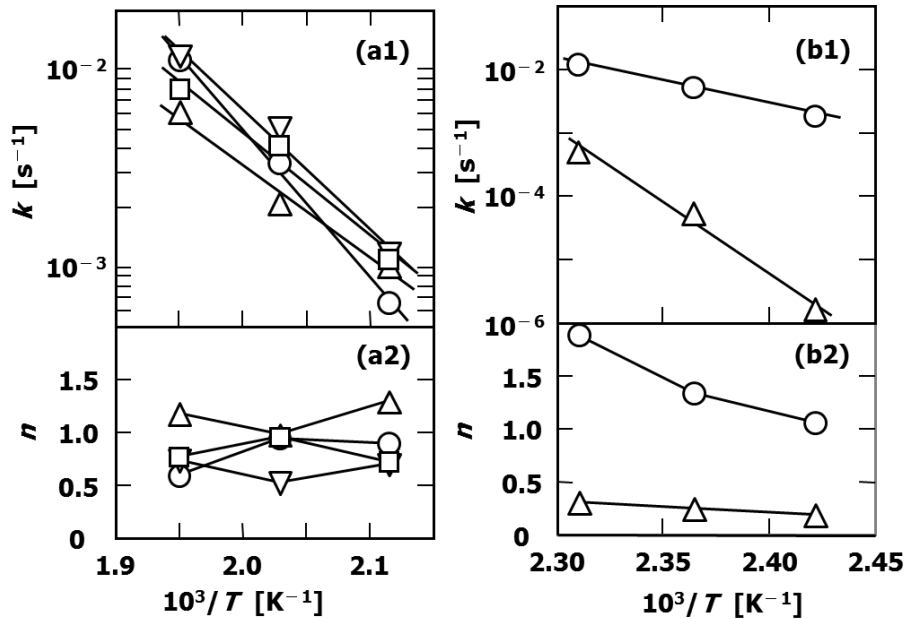


Figure 1-6. Temperature dependencies of (a1 and b1), the rate constant, k , and (a2 and b2), the shape constant, n , for the degradation of (a1 and a2) (Δ) arabinose, ($\circ$) xylose, (∇) ribose, and ($\square$) lyxose, and (b1 and b2) ($\circ$) glucuronic and (Δ) galacturonic acids.

It has been reported that the enthalpy-entropy compensation held for the degradation of hexoses in subcritical water (Haghighat Khajavi *et al.*, 2005). Equation (1-3) is one of the expressions describing the compensation (Exner, 1964; Leffler, 1955):

$$E = RT_{\beta} \ln k_0 + \gamma \quad (1-3)$$

where T_{β} is a parameter called the isokinetic temperature and γ is a constant. Figure 1-7 shows the plots of the E values versus the natural logarithms of the k_0 values for the degradation of the pentoses, hexouronic acids and hexoses. The E and k_0 values for the hexoses were cited from our previous study (Haghighat Khajavi *et al.*, 2005). The plots for the pentoses and hexoses lie on a straight line, and the T_{β} value was estimated to be 553 K (280°C) from the slope of the line. However, the plots for the hexouronic acids deviated from the line. The E and k_0 values for the galacturonic acid were very high. The plot for the substrate was beyond the figure, and was not along the line. These facts indicated that the degradation of the pentose and hexoses proceeded through essentially the same mechanism, but that the hexouronic acids were degraded through a mechanism different from that for the pentose and hexoses.

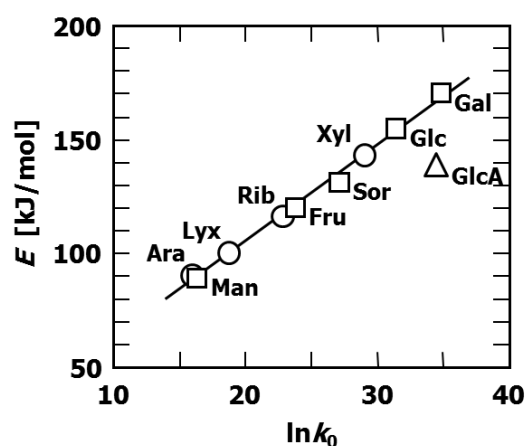


Figure 1-7. Relationships between the activation energy E and the natural logarithm of the frequency factor k_0 for the degradation of (○) pentoses, (△) hexouronic acids, and (□) hexoses in subcritical water. The E and k_0 values were cited from our previous study (Haghighat Khajavi *et al.*, 2005). The pentoses, hexouronic acids and hexoses are represented by the first three letters of their names, except for glucose; Ara: arabinose, Lyx: lyxose; Rib: ribose, Xyl: xylose; GlcA: glucuronic acid; Fru: fructose; Gal: galactose; Glc: glucose; Man: mannose; and Sor: sorbose. The plot for the galacturonic acid was beyond the figure.

1.3.4 Yield of furfural

Furfural is one of breakdown products of the pentoses (Parajó *et al.*, 1993; Chotěborská *et al.*, 2004; Rocha *et al.*, 2004). Figure 1-8 shows the relationships of the concentration between the consumed substrate and furfural formed from the pentoses. Although the plots have a high scatter, they roughly lie on a straight line having the slope of unity. This fact indicated that the amount of produced furfural was proportional to that of the consumed substrates. The yield was estimated on a molar basis to be 0.3 from the line for all the pentoses at any temperature.

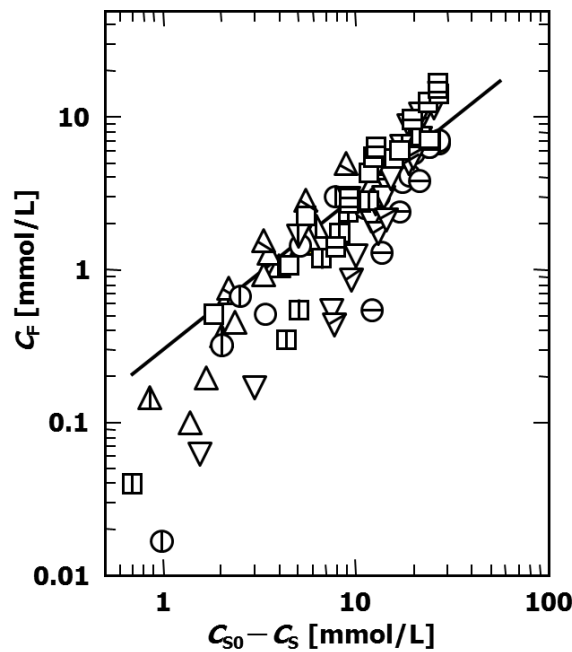


Figure 1-8. Conversion of (Δ) arabinose, ($\circ$) xylose, (∇) ribose, and ($\square$) lyxose to furfural in subcritical water at (with axial bar) 200°C, (without bar) 220°C, and (with horizontal bar) 240°C. C_{S0} , C_S , and C_F are the same as those in Fig. 1-3.

1.3.5 Formation of acidic compounds

The pH of the reactor effluent decreased as the degradation of pentoses proceeded. This fact indicated that acidic compounds were formed during the degradation. The pH values of the reactor effluent are plotted versus the concentration of the consumed substrate for all the pentoses in Fig. 1-9. As the substrate was consumed, the pH decreased. Although the plots have a high scatter, they also lie roughly on the line having the slope of unity on a semi-logarithmic scale. The pH value is a common logarithm of the concentration of the

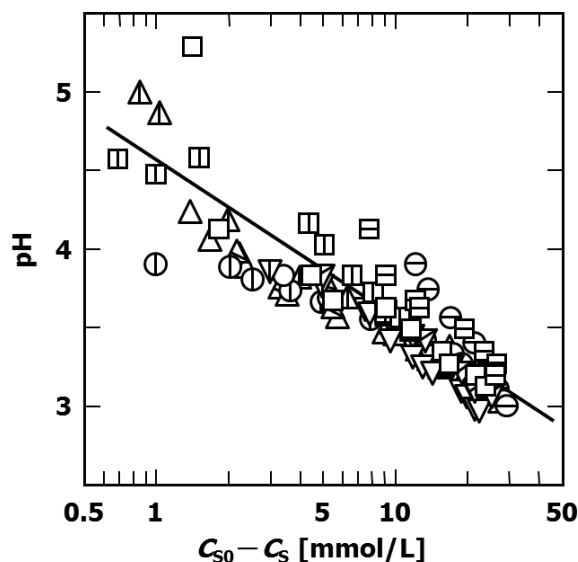


Figure 1-9. Relationship between the pH of the reactor effluent and the concentration of consumed substrates. The symbols are the same as those in Fig. 1-8.

hydrogen ion. Therefore, the slope of unity suggested that the concentration of the acidic compounds produced by the degradation of the pentoses was proportional to the concentration of the consumed substrates.

1.4 Conclusion

The degradation of arabinose, xylose, ribose, and lyxose in subcritical water was measured at different temperatures as well as that of the glucuronic and galacturonic acids. Their degradation processes could be expressed by the Weibull model. The susceptibility to the degradation depended on the pentose type. The hexouronic acids were degraded at temperatures lower than the pentoses. The enthalpy-entropy compensation held for the degradation of the pentoses and hexose, but not for the degradation of the hexouronic acids. Furfural was formed through the degradation of the pentose at the molar yield of *ca.* 0.3. Acidic compounds were also produced in proportion to the amount of the consumed substrates.

Chapter 2

Isomerization of Hexoses in Subcritical Water

2.1 Introduction

There are many reports on the hydrolysis and degradation of organic compounds in subcritical water, e.g., the hydrolysis of cellulose (Sasaki *et al.*, 1998; Sasaki *et al.*, 2000; Ehara and Saka, 2002), hydrolysis of plant oil (Holliday *et al.*, 1997), degradation of polycyclic aromatic hydrocarbons (Miller *et al.*, 1998), and decomposition of saccharides (Oomori *et al.*, 2004; Haghighat Khajavi *et al.*, 2005; Haghighat Khajavi *et al.*, 2006). The reactions of other types also occur in subcritical water. Sasaki *et al.* (2002) reported the retro-aldol condensation of glucose. We found that angiotensin II condensed with a dicarboxylic acid to form an amide bond (Asano *et al.*, 2005). Holliday *et al.* (1997) reported the isomerization of linolenic acid to its isomers during the hydrolysis of vegetable oils in subcritical water. The isomerization of glucose to fructose in sub- and supercritical waters is more prevalent than its reverse reaction (Kabyemela *et al.*, 1997; Strokol *et al.*, 2004; Watanabe *et al.*, 2005a; Watanabe *et al.*, 2005b). Strokol *et al.* (2004) pointed out the mutual conversion among mannose, glucose and fructose at 340°C and 27.5 MPa.

In this study, the isomerizations of mannose, fructose, and glucose in subcritical water are examined at relatively low temperatures (180 to 240°C), and their kinetics are discussed to investigate the catalytic properties of subcritical water.

2.2 Materials and Methods

2.2.1 Materials

D-(+)-Glucose, D-(-)-fructose, and D-(+)-mannose of analytical grade were purchased from Wako Pure Chemical Industries, Osaka, Japan.

2.2.2 Isomerization in subcritical water

The isomerization of mannose, fructose, or glucose were measured using a continuous flow system, which was similar to that used for degradation of pentose or hexouronic acid in the subcritical water (section 1.2.2). Each monosaccharide was dissolved in distilled water at

a concentration of 0.5, 2.0, or 5.0% (w/v). The reaction temperature was regulated at a specific value between 180 and 240°C.

The effluent was analyzed by high performance liquid chromatograph (HPLC) consisting of a pump (uf-3000 4S; Shimadzu, Tokyo, Japan), an Asahipak NH₂P-50 4E column (4.6 mm I.D. × 250 mm; Asahi Kasei Corporation, Tokyo, Japan), and an evaporative light-scattering detector (500 ELSD; Alltech, Deerfield, IL, USA) to determine the remaining substrate and isomerization products. The column was maintained at 30°C in a column oven. The eluent was a mixture of acetonitrile and water (80/20, v/v) flowing at the rate of 1.0 mL/min.

2.3 Results and Discussion

2.3.1 Treatment of mannose, fructose, and glucose in subcritical water

Figures 2-1(a) to (c) show the fractions of the remaining mannose and produced fructose and glucose, respectively, at 220°C at different residence times. The concentration of each hexose is normalized by the mannose concentration in the feed solution. The mannose concentration was lower at the longer residence time. There was no correlation between mannose concentration and disappearance. Mannose was isomerized to both fructose and glucose. The fructose and glucose concentrations increased with increasing residence time, peaked, and then decreased due to product degradation. The maximum conversions of the fructose and glucose were about 7% and 3%, respectively. The amounts of produced fructose and glucose were much lower than that of the lost mannose, indicating that most of the mannose was converted to 5-hydroxymethyl- 2-furaldehyde (HMF), acidic compounds, or other compounds (Haghighat Khajavi *et al.*, 2005). The degradation of mannose and its isomerization to fructose and glucose proceeded in parallel; the produced fructose and glucose were also consecutively degraded.

Fructose at concentrations of 0.5, 2.0, or 5.0% (w/v) were treated at 220°C at different residence times (Fig. 2-2). Fructose disappeared faster at the higher concentrations, although the reason is still unknown. Concentrations of mannose and glucose produced by the isomerization of the fructose were very low.

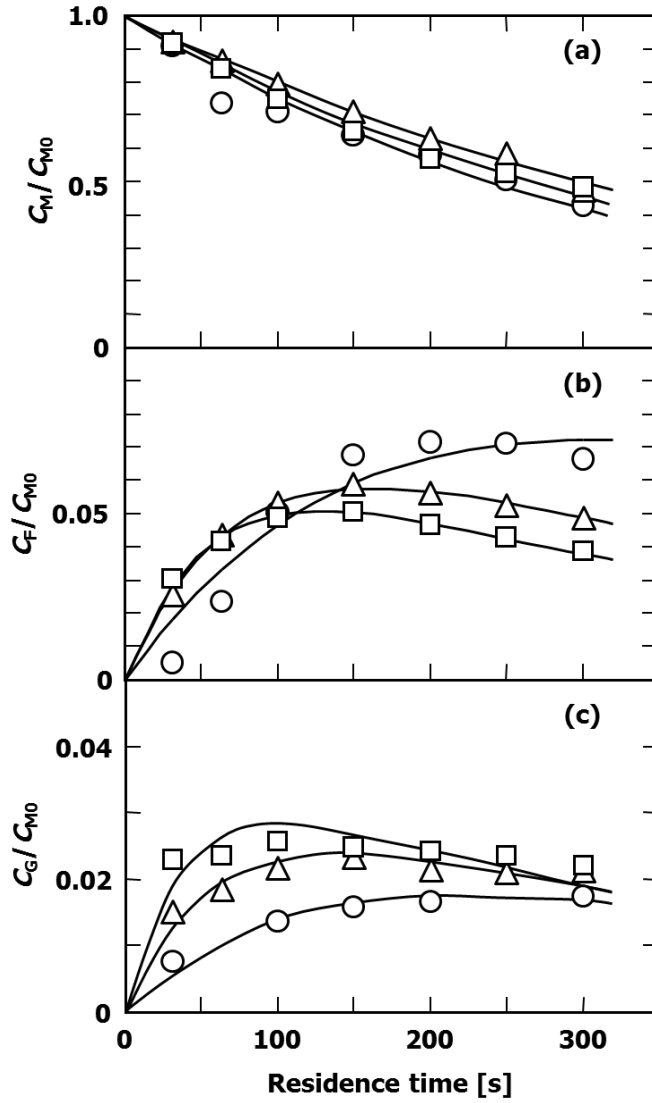


Figure 2-1. Changes in the concentration of (a) mannose, (b) fructose, and (c) glucose at 220°C and various residence times. The feed solutions were (○) 0.5% (w/v), (△) 2.0%, and (□) 5.0% mannose. The mannose, fructose, and glucose concentrations, C_M , C_F , and C_G , were normalized by the mannose concentration in the feed, C_{M0} .

Figures 2-3 (a) and (b) show the changes in the concentration of the glucose and fructose, respectively, when glucose was treated in the subcritical water at 220°C. Fructose was produced by the isomerization of glucose and then degraded at long residence times. No formation of mannose from glucose was observed.

2.3.2 Kinetic analysis

We applied the Weibull equation to the degradation processes of hexoses in subcritical water (Haghighat Khajavi *et al.*, 2005). The shape constant, which is a parameter of the

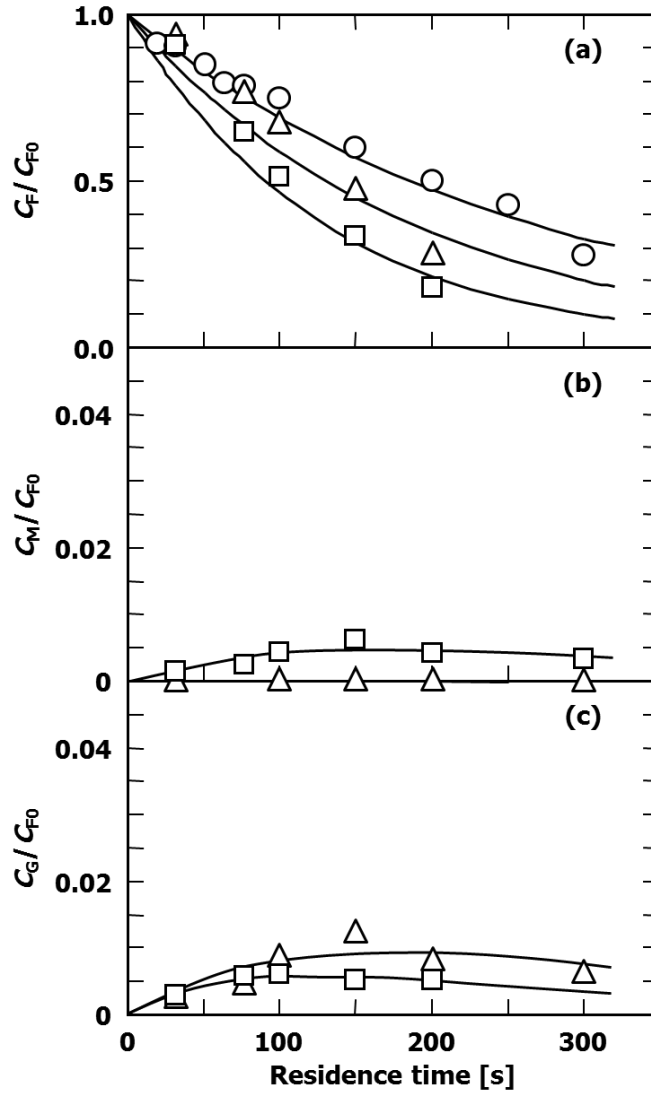


Figure 2-2. Changes in the concentration of (a) fructose, (b) mannose, and (c) glucose at 220°C and various residence times. The feed solutions were (○) 0.5% (w/v), (△) 2.0%, and (□) 5.0% fructose. The fructose, mannose, and glucose concentrations, C_F , C_M , and C_G , were normalized by the fructose concentration in the feed, C_{F0} .

equation, was nearly 1 for every hexose. This indicates that the disappearance of each hexose can be approximately expressed by first-order kinetics. We assumed that both the degradation and isomerization of any hexose can be expressed by first-order kinetics.

When considering the degradation on hexose i and its isomerization to hexoses j and k , each process is assumed to obey first-order kinetics. The hexoses j and k are also degraded according to first-order kinetics. The isomerization of hexose j or k to another hexose k or j is assumed to be negligible. Based on these assumptions, the mass balances of the hexoses i , j and k in the steady-state are given as follows:

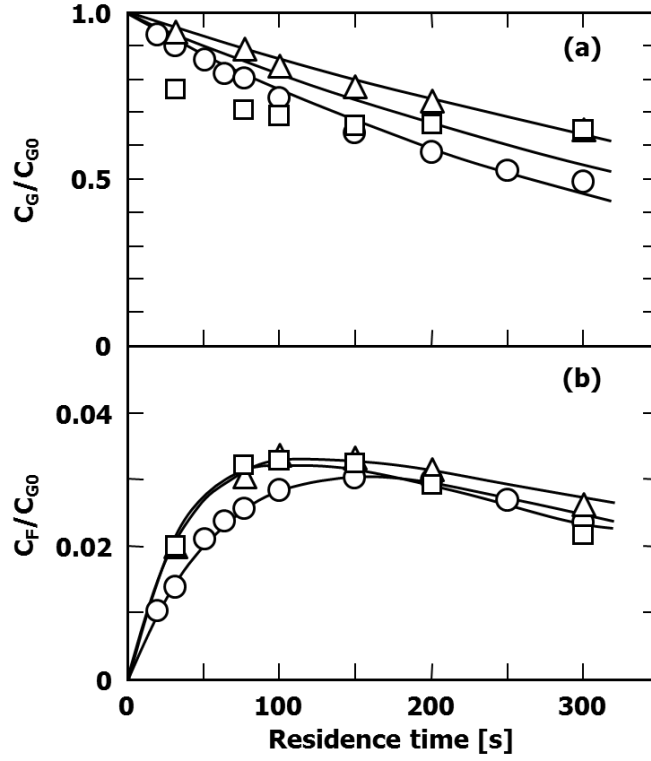


Figure 2-3. Changes in the concentration of (a) glucose and (b) mannose at 220°C and various residence times. The feed solutions were (○) 0.5% (w/v), (△) 2.0%, and (□) 5.0% glucose. No formation of mannose was observed. The glucose and fructose concentrations, C_F and C_G , were normalized by the glucose concentration in the feed, C_{G0} .

$$dC_i / d\tau = -(k_{id} + k_{i \rightarrow j} + k_{i \rightarrow k})C_i = -k_{it}C_i \quad (2-1)$$

$$dC_m / d\tau = k_{i \rightarrow m}C_i - k_{md}C_m \quad (m = j \text{ or } k) \quad (2-2)$$

where C is the concentration, τ is the residence time, and k is the rate constant for the first-order kinetics. The subscripts d and $i \rightarrow j$ or $i \rightarrow k$ indicate the degradation and isomerization of hexose i to hexose j or k , respectively. k_{it} is the rate constant for the overall disappearance of hexose i , and $k_{it} = k_{id} + k_{i \rightarrow j} + k_{i \rightarrow k}$. Equations (2-1) and (2-2) are analytically solved under the initial conditions of $C_i = C_{i0}$ and $C_m = 0$ ($m = j$ or k), respectively, at $\tau = 0$ to give the following equations.

$$C_i / C_{i0} = e^{-k_{it}\tau} \quad (2-3)$$

$$\frac{C_m}{C_{i0}} = \frac{k_{i \rightarrow m}}{k_{md} - k_{it}} (e^{-k_{it}\tau} - e^{-k_{md}\tau}) \quad (m = j \text{ or } k) \quad (2-4)$$

The rate constants, k_{it} , $k_{i \rightarrow m}$, and k_{md} , were evaluated to minimize the sum of the residuals square between the experimental and calculated C values using the Solver of Microsoft

Table 2-1. Rate constants estimated for the degradation and isomerization of mannose, fructose, and glucose at 220°C.

Substrate	Concentration [% (w/v)]	k_{Mt}	k_{Ft}	k_{Gt}	k_{Md}	k_{Fd}	k_{Gd}	$k_{M \rightarrow F}$	$k_{M \rightarrow G}$	$k_{F \rightarrow M}$	$k_{F \rightarrow G}$	$k_{G \rightarrow F}$
Mannose	0.5	2.86×10^{-3}			1.99×10^{-3}	3.76×10^{-3}	6.81×10^{-3}	6.48×10^{-4}	2.22×10^{-4}			
	2.0	2.33×10^{-3}			7.02×10^{-4}	1.31×10^{-2}	1.60×10^{-2}	1.10×10^{-3}	5.28×10^{-4}			
	5.0	2.62×10^{-3}			5.04×10^{-4}	1.72×10^{-2}	2.43×10^{-2}	1.22×10^{-3}	8.96×10^{-4}			
Fructose	0.5		3.71×10^{-3}									
	2.0		5.31×10^{-3}		9.58×10^{-3}	5.14×10^{-3}	6.51×10^{-3}			1.84×10^{-5}	1.49×10^{-4}	
	5.0		7.66×10^{-3}		4.58×10^{-3}	7.37×10^{-3}	9.16×10^{-3}			1.53×10^{-4}	1.38×10^{-4}	
Glucose	0.5			2.61×10^{-3}		1.35×10^{-2}						6.05×10^{-4}
	2.0			1.52×10^{-3}		2.32×10^{-2}						9.32×10^{-4}
	5.0			2.05×10^{-3}		2.48×10^{-2}						1.00×10^{-3}
Average		2.60×10^{-3}	5.56×10^{-3}	2.06×10^{-3}	3.47×10^{-3}	1.35×10^{-2}	1.26×10^{-2}	9.89×10^{-4}	5.49×10^{-4}	8.57×10^{-5}	1.44×10^{-4}	8.46×10^{-4}
Standard deviation		2.65×10^{-4}	1.99×10^{-3}	5.45×10^{-4}	3.78×10^{-3}	7.91×10^{-3}	7.60×10^{-3}	3.02×10^{-4}	3.37×10^{-4}	9.52×10^{-5}	7.78×10^{-6}	2.11×10^{-4}

The units are s^{-1} for all the rate constants.

Excel[®]. The curves in Figs. 2-1 to 2-3 are drawn using the rate constants, which are listed in Table 2-1. A rate constant was evaluated at different concentrations and for different reaction paths. The value of the rate constant is expressed by the mean and standard deviation.

The rate constant for overall disappearance was the highest for the fructose among the tested hexoses. The smallest k_{id} value for mannose indicates that mannose is the most resistant to degradation among the hexoses. Among the rate constants for the isomerization, $k_{M \rightarrow F}$ was the highest, where the subscripts M and F indicate mannose and fructose, respectively. That is, mannose is most easily isomerized to fructose in subcritical water among the mutual isomerizations of the hexoses.

2.3.3 Temperature dependence of isomerization of mannose

Mannose, the concentration of which was 0.5% (w/v), was treated in the subcritical water at different temperatures from 180°C to 240°C (Fig. 2-4). The rate constants, k_{Mt} , $k_{M \rightarrow F}$, $k_{M \rightarrow G}$, k_{Fd} , and k_{Gd} (G: glucose), were estimated at each temperature by the above-mentioned method. The curves in Fig. 2-4 were drawn using the rate constants. The k_{Md} value was calculated from $k_{Mt} - k_{M \rightarrow F} - k_{M \rightarrow G}$. The rate constants are plotted versus the reciprocal of the absolute temperature in Fig. 2-5. The plots for each rate constant gave a straight line on a semi-logarithmic scale, indicating that the temperature dependence of the rate constant can be expressed by the Arrhenius equation: where E_n is the activation energy, k_{n0} is the frequency factor, R is the gas constant, and T is the absolute temperature. The E_n and k_{n0} values for each process were calculated from this line. The activation energy for the isomerization of mannose to fructose, $E_{M \rightarrow F}$, was 106 kJ/mol, and was two times greater than that for the isomerization of mannose to glucose, $E_{M \rightarrow G}$.

The E_n values were plotted versus the natural logarithms of k_{n0} (Fig. 2-6). Because the plots lie on a straight line and Equation (2-6) is one of the expressions for enthalpy-entropy compensation (Exner, 1964; Leffler, 1955); the compensation is maintained for the

$$k_n = k_{n0} \exp(-E_n / RT) \quad (n = Mt, Md, Fd, Gd, M \rightarrow F, \text{ or } M \rightarrow G) \quad (2-5)$$

degradation and isomerization processes:

$$E_n = RT_\beta \ln k_{n0} + \gamma \quad (2-6)$$

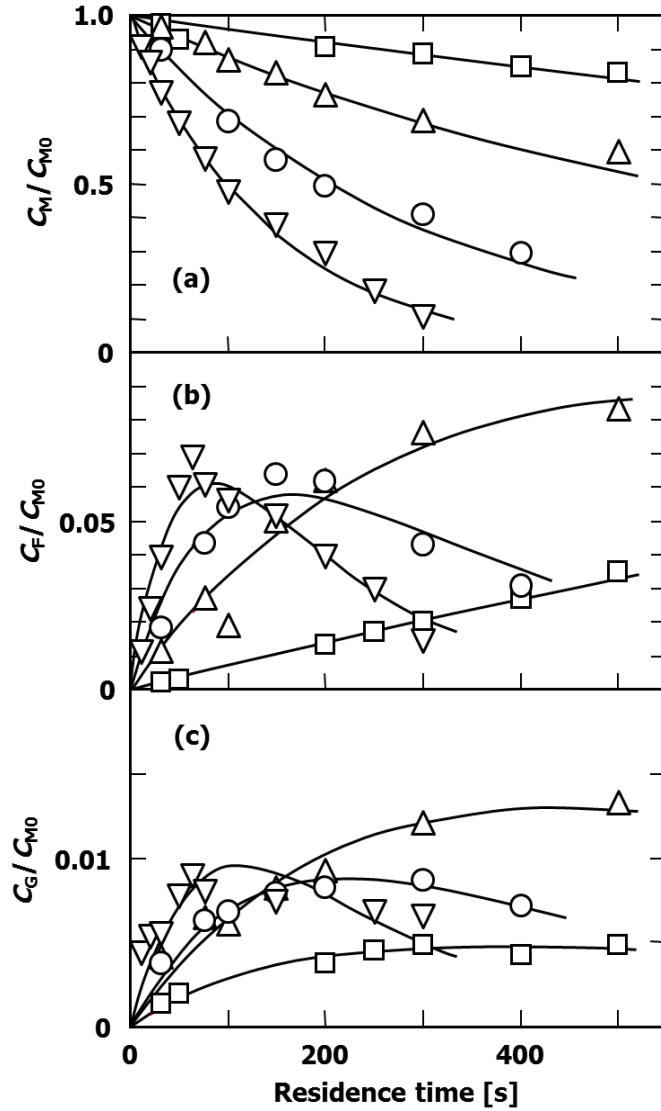


Figure 2-4. Changes in the concentration of (a) mannose, (b) fructose, and (c) glucose for the treatment of the 0.5% (w/v) mannose solution at ($\square$) 180°C, ($\triangle$) 200°C, ($\circ$) 220°C, and (∇) 240°C. The mannose, fructose, and glucose concentrations, C_M , C_F , and C_G , were normalized by the mannose concentration in the feed, C_{M0} .

where T_β is a parameter called the isokinetic temperature, and γ is a constant. The T_β was evaluated to be 153°C. At this temperature, all processes are predicted to proceed at the same rate.

2.4 Conclusion

Isomerization of mannose to fructose occurred most frequently, followed by mannose to glucose; no isomerization of glucose to mannose was observed. The temperature dependencies of mannose disappearance, the isomerization of mannose to fructose and

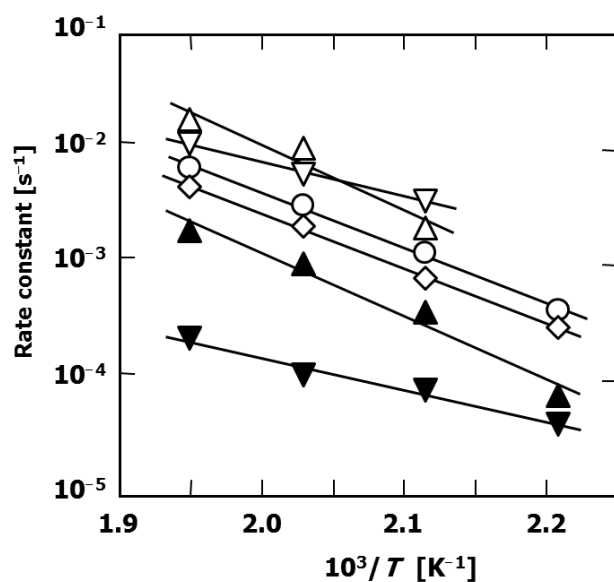


Figure 2-5. Arrhenius plots for the rate constants, ($\circ$) k_{Mb} , ($\diamond$) k_{Md} , ($\triangle$) k_{Fd} , (∇) k_{Gd} , ($\blacktriangle$) $k_{M \rightarrow F}$, and ($\blacktriangledown$) $k_{M \rightarrow G}$.

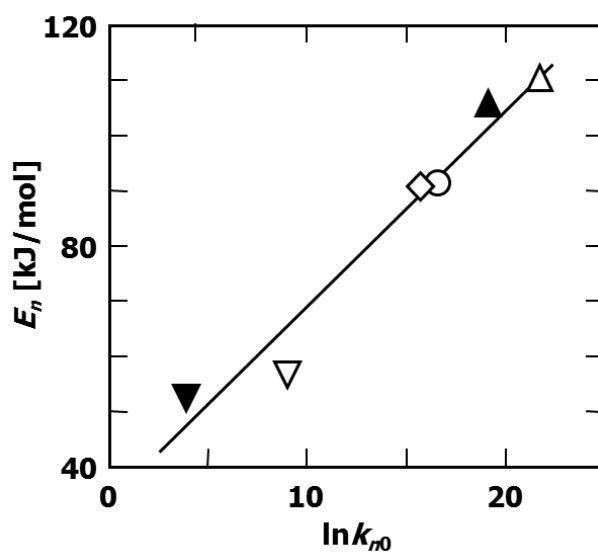


Figure 2-6. Enthalpy-entropy compensation for the degradation and isomerization of hexoses. The units of the frequency factor, k_{r0} , are s^{-1} . The symbols are the same as in Fig. 2-5.

glucose, and the resulting degradation of the produced fructose and glucose were observed. It was found that the enthalpy-entropy compensation held for the processes of degradation and isomerization of hexoses in subcritical water.

Chapter 3

Conversion of Linoleic Acid to Its Conjugated Isomers in Subcritical Water

3.1 Introduction

There are some reports of the hydrolysis and degradation of organic compounds in subcritical water, such as cellulose (Sasaki *et al.*, 1998; Sasaki *et al.*, 2000; Ehara and Saka, 2002;), vegetable oil (Holliday *et al.*, 1997), polycyclic aromatic hydrocarbon (Yang and Hilderbrand, 2006), and saccharides (Oomori *et al.*, 2004; Haghighat Khajavi *et al.*, 2005) as well as condensation (Sasaki *et al.*, 2002; Asano *et al.*, 2005). The isomerizations of monosaccharide (Kabyemela *et al.*, 1997) and linolenic acid (Holliday *et al.*, 1997) in subcritical water have also been reported. The isomerization of linoleic acid into its conjugated isomers, to which much attention has been paid due to their physiological functionalities (Fritsche and Steinhart, 1998), has been reported in the absence of water (Destailats and Angers, 2005).

In this study, it was determined whether the isomerization of linoleic acid to its conjugated isomers occurs in subcritical water or not, and what type of isomer is apt to be formed if the isomerization occurs.

3.2 Materials and Methods

3.2.1 Materials

Palmitic acid (> 95%), which is used as the internal standard in gas chromatographic analysis, and linoleic acid (purity > 90%) were purchased from Tokyo Kasei Kogyo, Tokyo, Japan. The *trans*10, *cis*12 and *cis*9, *trans*11 conjugated linoleic acids, which are abbreviated t10,c12 and c9,t11 CLAs, respectively, and the purities of which were both > 96%, were purchased from the Cayman Chemical Co., Ann Arbor, MI, USA. The (trimethylsilyl) diazomethane solution of 2.0 mol/L in hexane, abbreviated TMSCHN₂, was purchased from Sigma Aldrich Japan, Tokyo.

3.2.2 Isomerization in subcritical water

Specified volumes of linoleic acid and distilled water, which had been sonically degassed,

were placed in a pressure-resistant vessel (Taiatsu Garasu, Osaka, Japan; inner volume, 5 mL). The volumes of linoleic acid and water were 0.1 mL and 2.0 mL, 1.0 mL and 1.0 mL, or 2.0 mL and 0.1 mL. Linoleic acid with no water was also tested. The CLAs are produced by the isomerization of linoleic acid in the presence of a dense solution of potassium hydroxide at ca. 180°C (Yang *et al.*, 2002). In this study, linoleic acid (1.0 mL) was mixed with a dilute KOH solution (0.01 mol/L, 1.0 mL) and then treated at 200°C. The vessel was set in a GC-17A or GC-7A oven (Shimadzu, Kyoto) regulated at a temperature from 200 to 320°C. After a specific time elapsed, the vessel was immersed in ice-water to stop the reaction.

The reaction mixture was then transferred to a glass vial. The vessel was rinsed with 10 mL of a chloroform-methanol mixture (2:1 by vol.) and the washing was also placed in the vial. One milliliter of 3 mol/L HCl was added to the vial, and distilled water was further added to make the volume of aqueous phase 3 mL. No water was added when the reaction was carried out using 2 mL of water. After the mixture was vigorously shaken for 30 s, it was centrifuged at 3000 rpm for 5 min. The chloroform phase of the lower layer was added to another small vial, and evaporated under reduced pressure. Fatty acids in the vial were methylesterified with 30 μ L of TMSCHN₂, 2.0 mL of benzene and 0.5 mL of methanol. After the solvent was evaporated under reduced pressure, the remainder was dissolved with 0.5 mL of hexane. The solution (1 μ L) was applied to a GC-14B gas chromatograph (Shimadzu) with a hydrogen ionization detector to determine the fatty acids in the reaction mixture. The separation column was a DB1 capillary column (0.32 mm ϕ $\times$ 30 m; J & W Scientific, Folsom, CA, USA). The injection, column and detector temperatures were 250°C, 220°C, and 270°C, respectively. The flow rates of carrier helium gas was 3 mL/min with a split ratio of 20:1, and the flow rate of makeup gas was 23.5 mL/min. The pressures of the hydrogen gas and air were 55 and 60 kPa, respectively.

3.3 Results and Discussion

Figures 3-1 (a) and (b) show the gas chromatograms for the mixture of linoleic acid (0.1 mL) and water (2.0 mL) and for the linoleic acid alone, respectively, treated at 200°C for 12 h. In both cases, small amounts of c9,t11 and t10,c12 CLAs were formed. They were identified

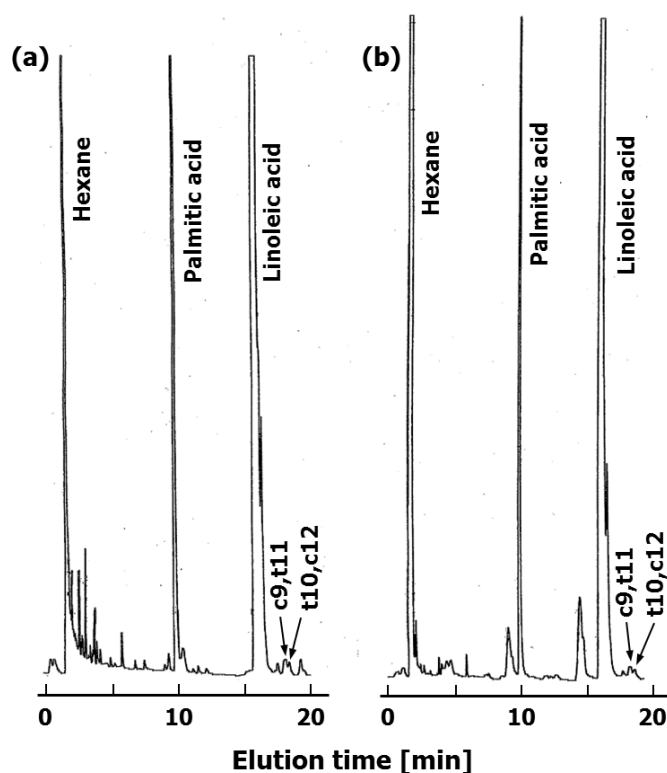


Figure 3-1. Gas chromatograms of (a) the mixture of linoleic acid and water at the volume ratio of 0.1 to 2.0 and (b) linoleic acid alone, which were treated at 200°C for 12 h.

by comparing the retention times with those of the reagent CLAs. Their molecular masses were also confirmed to coincide with those of the respective CLAs. The amount of c9,t11 CLA was greater than that of t10,c12 CLA.

The effect of temperature on the formation of the CLAs at 12 h was examined for linoleic acid alone and for 0.1 mL of linoleic acid mixed with 2.0 mL of water (Fig. 3-2). The ordinate indicates the fraction of the sum of c9,t11 and t10,c12 CLAs to the total linoleic acids (C18:2; non-conjugated plus conjugated). More CLAs were formed at the higher temperatures. The presence of water did not influence the formation of the CLAs. The inset of Fig. 3-2 shows the temperature dependence of the ratio of the t10,c12 isomer to the c9,t11 one for the linoleic acids. The temperature scarcely affected the ratio as well as the presence of water, and the ratio was roughly 0.6 at any temperature.

The changes with time in the fraction of the sum of the c9,t11 and t10,c12 CLAs to the total linoleic acids were examined for the linoleic acids mixed with water at various ratios or with 0.01 mol/L KOH (Fig. 3-3). The isomerization seemed to be a very slow reaction and

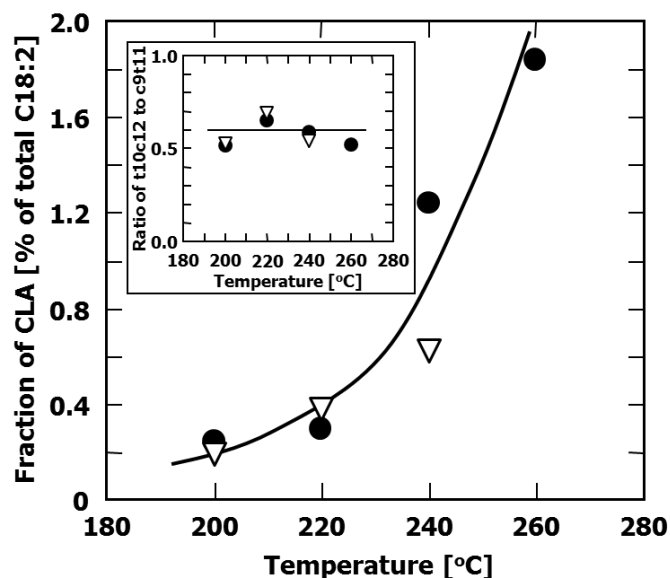


Figure 3-2. Temperature dependence of the fraction of conjugated isomers (CLA) in the sum of unconjugated and conjugated linoleic acids for (●) linoleic acid alone and (▽) the mixture of linoleic acid and water at the volume ratio of 0.1 to 2.0. Inset: The ratio of t10,c12 CLA to c9,t11 CLA at various temperatures. The symbols are the same as in the main figure.

required a long time to reach equilibrium although the dispersion of the data was significant. The volume ratio of linoleic acid to water scarcely affected the isomerization of the linoleic acid to the CLAs. The yield of the CLAs from linoleic acid mixed with 0.01 mol/L KOH was lower than that of the CLAs from the linoleic acid mixed with or without water although the reason remains unclear.

It has been reported that CLAs of many types are formed through the KOH-catalyzed isomerization of linoleic acid and that the c9,t11 and t10,c12 isomers are the two major components among the CLAs (Yang *et al.*, 2002). Under our analytical conditions, only the c9,t11 and t10,c12 isomers could be determined. In order to estimate the selectivity during the isomerization, the fraction of the t10,c12 isomer is plotted versus the fraction of the c9,t11 one in Fig. 3-4 for the results shown in Fig. 3-3. All the plots lie on a line with a slope of 0.6. This indicates that the c9,t11 and t10,c12 isomers are formed at the molar ratio of 1.0:0.6 under the tested conditions. The ratio was identical to the value shown in the inset of Fig. 3-2. However, the ratio in the KOH-catalyzed isomerization of linoleic acid was estimated to be about 1:0:1.15 at 180°C from the HPLC chromatogram in the literature (Yang *et al.*, 2002). Although the reason for the discrepancy still remains unclear, a possible reason is that the

equilibrium was not attained because of the extremely slow progress of the isomerization in our study.

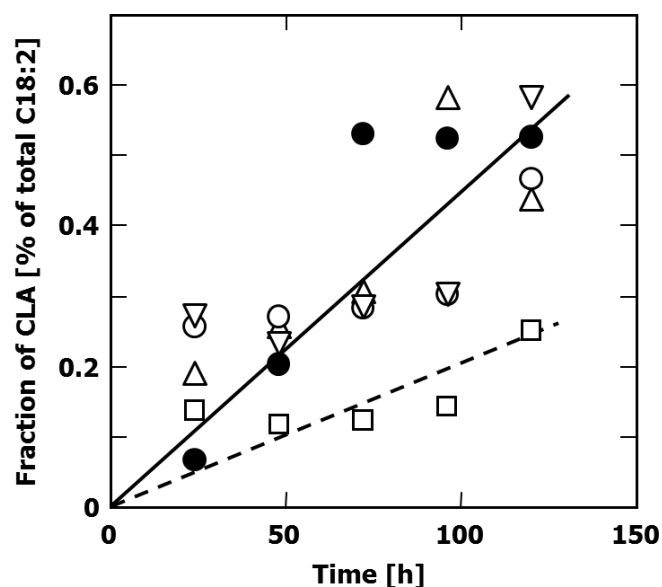


Figure 3-3. Transient changes at 200°C in the fraction of CLAs in the sum of unconjugated and conjugated linoleic acids. Samples were (●) linoleic acid alone, linoleic acids mixed with water at the volume ratios of (△) 2.0 to 0.1, (○) 1.0 to 1.0, and (▽) 0.1 to 2.0, and (□) linoleic acid mixed with 0.01 mol/L KOH at the volume ratio of 1.0 to 1.0.

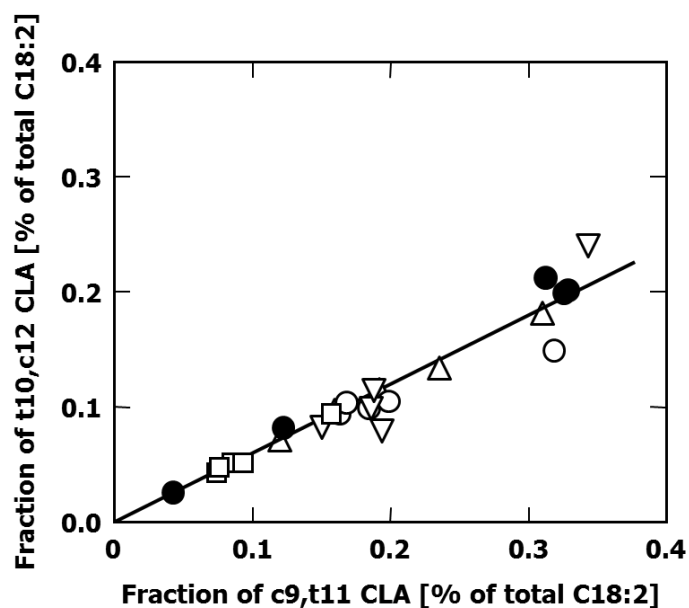


Figure 3-4. Relationship between the fraction of c9,t11 isomer and that of t10,c12 one during the isomerization of linoleic acid at 200°C. The symbols are the same as in Fig. 3-3.

3.4 Conclusion

Linoleic acid was heated in the presence or absence of water to examine the possibility of its conversion to conjugated isomers. The conversion occurred at very low yields, and the major products were the c9,t11 and t10,c12 conjugated linoleic acids. The ratio of the t10,c12 isomer to the c9,t11 one was about 0.6, and it did not depend on both the temperature and the ratio of linoleic acid to water.

Chapter 4

Kinetics of the Disappearance of *N*-Acetyl-D-glucosamine in Subcritical Aqueous Ethanol

4.1 Introduction

N-Acetyl-D-glucosamine (GlcNAc) is a constituent of chitin, which comprises the outer skin of crustaceans and insects. GlcNAc and glucosamine prepared from crab shells are used as components of health food supplements. Subcritical water treatment of the crab skin has been reported to produce chitin oligomers (Osada *et al.*, 2015). To elucidate the phenomena occurring during the treatment, we investigated the degradation kinetics in subcritical water (Wang *et al.*, 2011).

Under subcritical conditions, a mixture of ethanol and water was more effective than water alone for the extraction of phenolic and antioxidative substances from defatted rice bran and rice straw (Chiou *et al.*, 2012; Tangkhavanich *et al.*, 2013). The isomerization of common saccharides to rare ones was promoted by the addition of ethanol to water (Gao *et al.*, 2015a; Gao *et al.*, 2016). Although the treatment of chitin in subcritical aqueous ethanol was not examined, similar treatment of GlcNAc and glucosamine should provide basic knowledge on the phenomena that occur during the treatment.

In this context, the disappearance of GlcNAc in subcritical aqueous ethanol was examined and kinetically analyzed.

4.2 Materials and Methods

4.2.1 Materials

GlcNAc (>98%) was supplied by Koyo Chemicals (Osaka, Japan). Ethanol was purchased from Wako Pure Chemical Industries (Osaka, Japan).

4.2.2 Treatment of GlcNAc in subcritical aqueous ethanol

GlcNAc was dissolved in 0% (water), 20%, 40%, 60%, or 80% (w/w) aqueous ethanol at a concentration of 0.5% (w/w). The feed solution was sonically degassed before treatment under subcritical conditions and then connected to a nitrogen gas bag to prevent redissolution

of atmospheric oxygen. The feed solution was delivered into a coiled stainless steel (SUS 316) tubular reactor (0.8 mm I.D. $\times$ 2.0 m length) immersed in a bath filled with an SRX 310 silicone oil (Toray-Dow-Corning, Tokyo, Japan) with a residence time of 20–240 s using an L-7100 HPLC pump (Hitachi, Tokyo, Japan). The residence time was calculated based on the inner diameter and length of the stainless steel tube and the density of the water-ethanol mixture under subcritical conditions according to our previous study (Wang *et al.*, 2011). The treatment was conducted at 190°C. The reactor effluent was directly introduced into a stainless steel tube (0.8 mm I.D. $\times$ 1.0 m length) immersed in an ice-water bath to terminate the reaction and then collected in a sampling vessel. The pressure inside the tube was regulated at 10 MPa using a back-pressure regulator (high-pressure adjustable BPR P-880; Upchurch, Washington, USA). The effluent aliquot (usually 0.70 mL) in the sampling vessel was evaporated under reduced pressure. The residue was dissolved in the same volume of distilled water to prepare the sample for HPLC analysis and pH measurement.

4.2.3 Determination of *N*-acetyl-D-glucosamine

The concentration of GlcNAc in the effluent was determined using an HPLC consisting of an L-7100 pump (Hitachi, Tokyo, Japan), a COSMOSIL Hilic column (3.0 mm I.D. $\times$ 150 mm, Nacalai Tesque, Kyoto, Japan), and an L-3350 refractometer (Hitachi, Tokyo, Japan). A mixture of 10 mmol/L ammonium acetate and acetonitrile (10/90, v/v) was used as the eluent at a flow rate of 0.4 mL/min. The column temperature was maintained at 30°C in an L-7300 column oven (Hitachi, Tokyo, Japan).

4.2.4 pH and absorption spectra measurements

The pH of the effluent was measured at room temperature using an F-14 pH meter (Horiba, Kyoto). The effluents were diluted 200 and 10 times using distilled water for measuring the ultraviolet (200–350 nm) and visible light (350–650 nm) absorption spectra, respectively, using a Multiskan GO Microplate spectrophotometer (Thermo Scientific, Vantaa, Finland).

4.3. Results and Discussion

4.3.1 Disappearance of *N*-acetyl-D-glucosamine in subcritical aqueous ethanol

First, 0.5% (w/w) GlcNAc samples dissolved in 0% to 80% (w/w) aqueous ethanol solutions were heated at 190°C, and the disappearance of GlcNAc in the reactor effluent was observed (Fig. 4-1). The rate of the disappearance of GlcNAc decelerated at higher ethanol contents. Because the disappearance of GlcNAc in subcritical water can be expressed using first-order kinetics (Wang *et al.*, 2011), the following kinetics were adopted to describe the changes in the concentration of GlcNAc in aqueous ethanol:

$$C/C_0 = \exp(-k\tau) \quad (4-1)$$

where k is the rate constant of the disappearance, C denotes the concentration of the remaining substrate (GlcNAc) in the reactor effluent, C_0 is the substrate concentration of the feed, and τ is the mean residence time of the substrate solution in the reactor. Plots of the fraction of the remaining substrate, i.e., C/C_0 , versus τ on the semi-logarithmic scale were

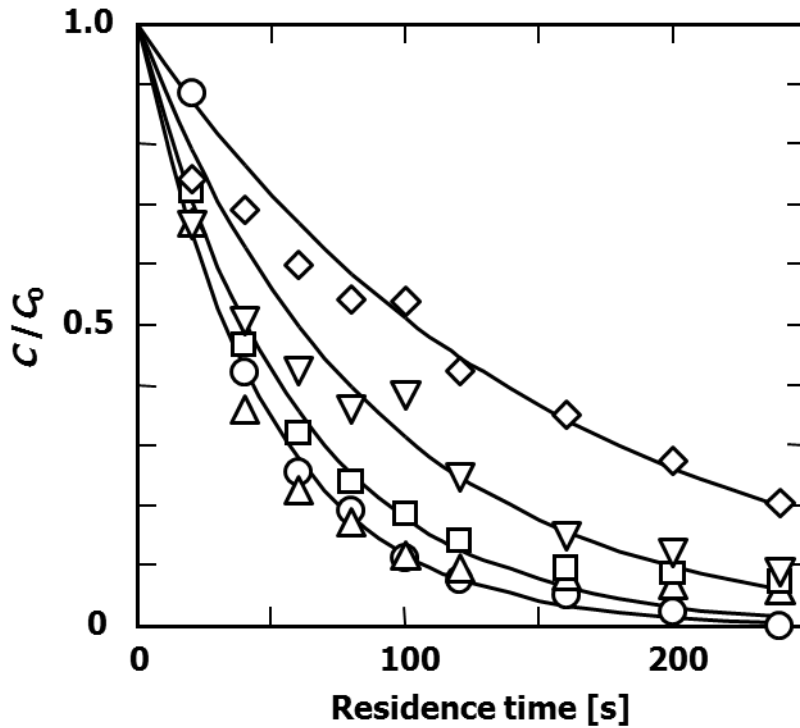


Figure 4-1. Disappearance of *N*-acetyl-D-glucosamine (GlcNAc) in subcritical aqueous ethanol at 190°C. The feed concentration of GlcNAc, C_0 , was fixed at 0.5% (w/w) in all aqueous ethanol solutions, and C represents the GlcNAc concentration in the reactor effluent. The ethanol content was (○) 0% (water), (△) 20%, (□) 40%, (▽) 60%, and (◇) 80% (w/w).

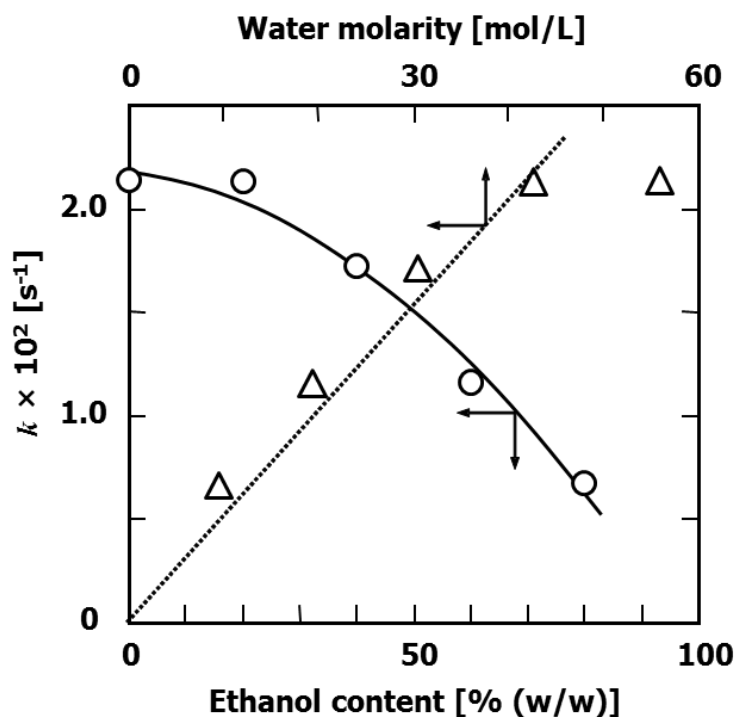


Figure 4-2. Dependence of the rate constant, k , for the disappearance of *N*-acetyl-D-glucosamine at 190°C on the (○) ethanol content and (△) water molarity.

linear, and k was estimated from the slope of the line at each ethanol content. The solid curves in Fig. 4-1 were drawn by substituting the estimated rate constants in Eq. (4-1). Figure 4-2 shows the dependence of the rate constant on the ethanol content. The rate constant was lower at higher ethanol contents. The water molarity was also lower at higher ethanol contents. The water molarity at room temperature was roughly evaluated assuming additivity of the volumes of water and ethanol. The rate constants were also plotted against water molarities (the upper abscissa) in Fig. 4-2. It was roughly proportional to water molarity, which indicates that water plays a major role in the disappearance of GlcNAc under subcritical conditions, while ethanol acts merely as a diluent. A reason for the deviation from linearity at the highest water molarity, which corresponds to water alone, remains unclear.

4.3.2 pH Changes during treatment

The pH values of the reactor effluents are shown in Fig. 4-3. The pH of the reactor effluent decreased as GlcNAc degraded. The rate of the change in pH with residence time

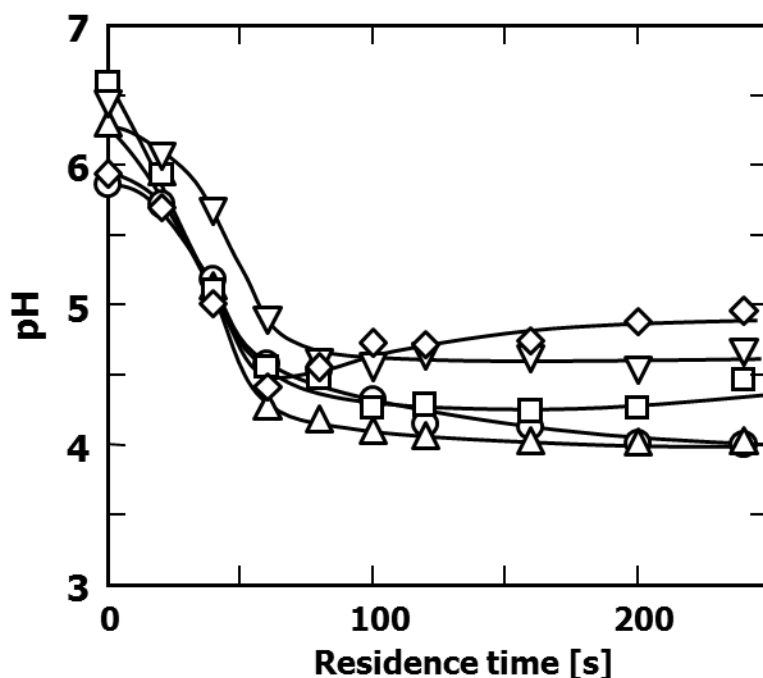


Figure 4-3. Changes in the pH of the reactor effluents for the treatment of *N*-acetyl-D-glucosamine at 190°C in subcritical fluids with different ethanol contents. The symbols are the same as those in Fig. 4-1.

decelerated at higher ethanol contents; this indicates that acidic compounds formed at lower ethanol contents and the concentration of hydrogen ion increased during treatment (Asghari and Yoshida, 2007; Kabyemela *et al.*, 1999; Jin *et al.*, 2004). However, the decrease in pH leveled off at a residence time of 60 s or longer. As shown in section 4.3.3, the ultraviolet absorption spectra of the reactor effluent suggest the formation of carboxylic acids. Liberation of the acetyl group from GlcNAc produces acetic acid and glucosamine, which generate a buffer system. This is likely the reason that the pH reaches a minimum value during the degradation of GlcNAc.

4.3.3 Absorption spectra

Figure 4-4 shows the ultraviolet absorption spectra from 200 to 350 nm for the reactor effluents with different residence times, which were diluted 200 times with water. In subcritical water (Fig. 4-4(a)), the absorbance near 230 nm increased rapidly at short residence times and maintained almost a constant value at longer residence times. The absorbance near 230 nm suggests the formation of carboxylic acids, and the increase in the

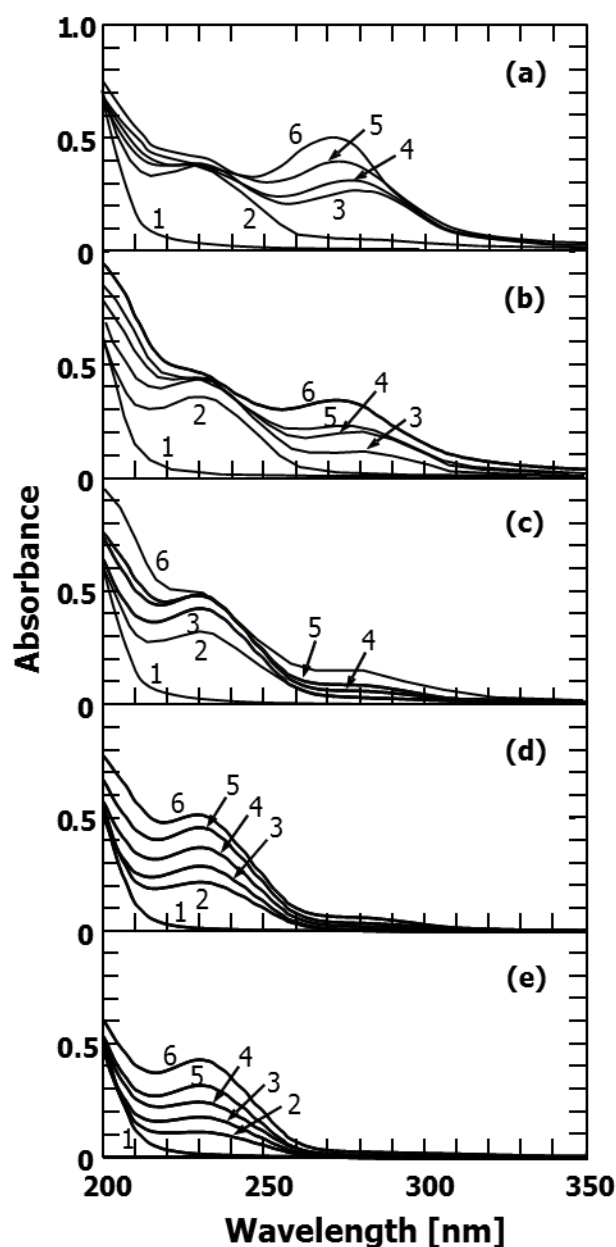


Figure 4-4. Ultraviolet absorption spectra of the reactor effluents after the treatment of *N*-acetyl-D-glucosamine at 190°C in subcritical fluids with different ethanol contents. The spectra were measured after 200 times dilution of the reactor effluents. The ethanol contents were (a) 0% (water), (b) 20%, (c) 40%, (d) 60%, and (e) 80% (w/w). The number symbols, 1–6, indicate residence times of 0 (feed solution), 40, 80, 120, 160, and 240 s, respectively.

absorbance observed near 270 nm suggests the formation of furfurals (Boopathy *et al.*, 1993). The increase in the absorbance near 230 nm was also observed in subcritical aqueous ethanol (Figs. 4-4(b)–(e)), although the rate of the increase was slower at higher ethanol contents. The increase in the absorbance near 270 nm was suppressed with increasing ethanol content. No

increase in the absorbance was observed in 80% (w/w) ethanol.

Figures 4-5(a) and (b) show the changes in the absorbances at 230 and 269 nm, respectively, with residence time. The rate of the increase of the intensity of the absorbance at 230 nm was rapid at short residence times and slower with lower ethanol content, i.e., higher water molarity. This indicates that water plays an important role in the degradation of GlcNAc. The absorbance reached a constant value of *ca.* 0.4 and then plateaued. The absorbance at 269 nm also increased faster at lower ethanol contents. A lag between the increase in the absorbance peaks at 230 and 269 nm, respectively, was observed; this is likely because

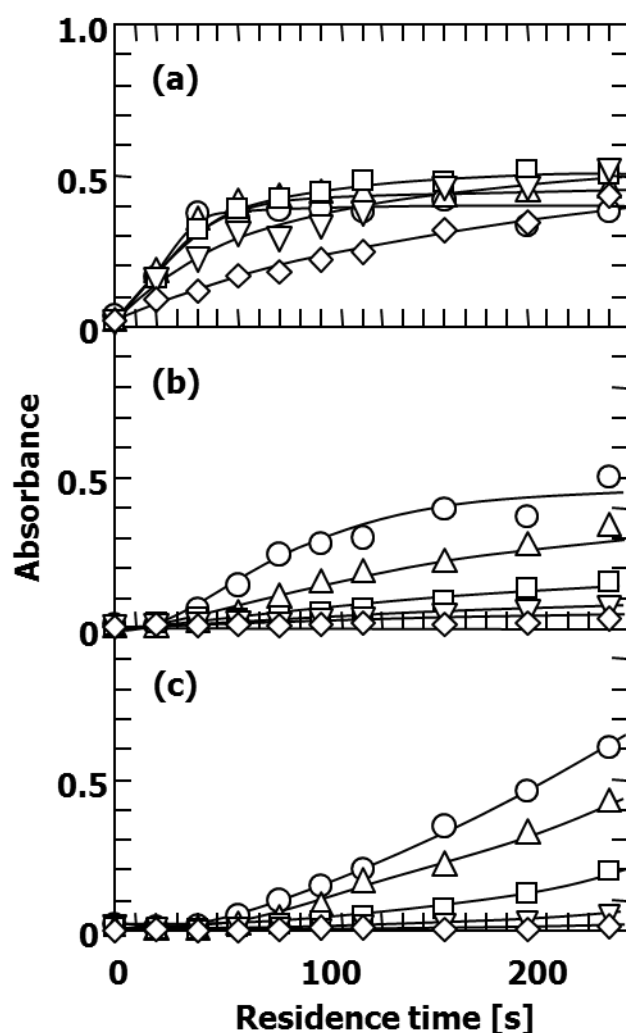


Figure 4-5. Changes in the absorbance at (a) 230, (b) 269, and (c) 400 nm of the reactor effluents from the treatment of *N*-acetyl-D-glucosamine in subcritical fluids with different ethanol contents at 190°C. The ultraviolet and visible light absorbances of the reactor effluent were measured after 200 and 10 times dilution with water, respectively. The symbols are the same as those in Fig. 4-1.

GlcNAc degrades to acetic acid and glucosamine, which is then further degraded to furfurals. The consecutive generation of furfurals results in the time lag in the increase of the absorbance at 269 nm.

The visible light spectra of the reactor effluents, which were diluted 10 times with water, were also measured. The spectra show a monotonic decrease in intensity with increasing wavelength. Figure 4-6 shows the spectra of the reactor effluents with a residence time of 240 s. The absorbance at any wavelength was higher for the reactor effluent in subcritical fluid with lower ethanol contents. Figure 4-5(c) shows the changes in the absorbance at 400 nm, which was selected as a representative wavelength for assessing the coloration of the reactor effluent in subcritical fluids with different ethanol contents. The coloration also lagged in its development, and no significant color development was observed in 80% (w/w) ethanol. Glucosamine, which was liberated from GlcNAc, undergoes the Maillard reaction to generate a yellow or brown color (Oyaizu, 1986). Therefore, the reason for the lag in color development is that it is generated in the second step or subsequent steps in the reaction. The greater color development in the fluid with lower ethanol content indicates that water also plays an important role in the coloration of the GlcNAc solution.

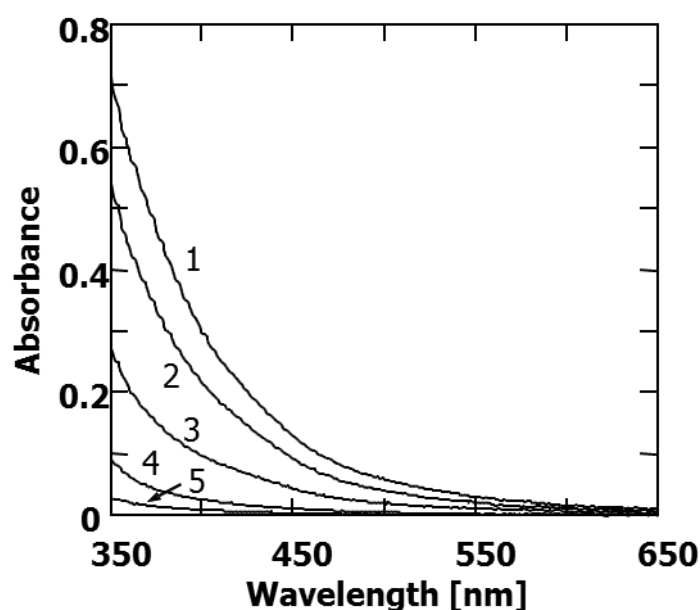


Figure 4-6. Visible light absorption spectra of the reactor effluents from treatments with a residence time of 240 s at 190°C in (1) water and (2) 20%, (3) 40%, (4) 60%, and (5) 80% (w/w) ethanol. The spectra were measured after 10 times dilution with water.

4.4 Conclusion

The disappearance of GlcNAc in all the aqueous ethanol fluids obeyed first-order kinetics, and the rate constant was roughly proportional to the water molarity of the aqueous ethanol; this indicates that water plays an important role in the disappearance of GlcNAc. The pH of the reaction mixture decreased as the reaction proceeded and then plateaued because of the formation of a buffer system by glucosamine and the acetic acid liberated from GlcNAc. The ultraviolet absorption spectra of the reactor effluents suggested the formation of both carboxylic acids and furfurals. The formation of furfurals and color development were suppressed at higher ethanol contents.

Chapter 5

Interconversion between D-Glucuronic Acid and D-Glucuronolactone in Subcritical Aqueous Ethanol

5.1 Introduction

D-Glucuronic acid (GlcA) is a hexouronic acid obtained through the hydrolysis of hemicellulose, which is a major component of biomass. GlcA is converted to D-glucuronolactone (GlcL) in subcritical water (Wang *et al.*, 2010). We previously reported that the addition of ethanol to water significantly promotes the isomerization of mono- and disaccharides under subcritical conditions (Gao *et al.*, 2014; 2015a; 2015b; 2015c; 2016). However, it remains unclear which reactions will occur during the treatment of GlcA in subcritical aqueous ethanol. In this context, the interconversion between GlcA and GlcL in subcritical aqueous ethanol is examined at relatively low temperatures (180°C or 200°C) and kinetically analyzed.

5.2 Materials and Methods

5.2.1 Materials

D-Glucuronic acid (GlcA, purity >98%) and ethanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). D-Glucurono-6,3-lactone (GlcL, purity >99%) was obtained from Sigma-Aldrich Japan (Tokyo).

5.2.2 Treatment of D-glucuronic acid or D-glucuronolactone in subcritical aqueous ethanol

A continuous flow-type reactor and procedures were the same as that described in Chapter 4. The temperature was regulated 180°C and 200°C. The effluent of a fixed volume (usually 0.20 mL) in a sampling vessel was evaporated under reduced pressure. The remainder was dissolved with distilled water of the same volume to prepare the sample for HPLC analysis.

5.2.3 HPLC analysis

The concentrations of GlcA and GlcL in the effluent were determined using an HPLC

consisting of a pump (L-7100, Hitachi, Tokyo, Japan), a COSMOSIL Hilic column (3.0 mm I.D. $\times$ 150 mm, Nacalai Tesque, Kyoto, Japan), and a refractometer (L-3350, Hitachi). A mixture of 10 mmol/L $\text{CH}_3\text{COONH}_4$ and acetonitrile (50/50, v/v) was used as the eluent at a flow rate of 0.4 mL/min. The column temperature was maintained at 30°C in an L-7300 column oven (Hitachi).

5.3. Results and Discussion

5.3.1 Treatment of D-glucuronic acid or D-glucuronolactone in subcritical aqueous ethanol at 180°C

The 0.5% (w/w) GlcA samples dissolved in 0% to 80% (w/w) aqueous ethanol solutions were heated at 180°C, and the disappearance of GlcA and the formation of GlcL were observed (Fig. 5-1). The disappearance of GlcA was decelerated at higher ethanol concentrations, while the effect of ethanol content on the formation of GlcL was complicated. The formation of GlcL was largely suppressed at 80% (w/w) ethanol. The formation of GlcL would proceed through acid-catalyzed lactonization. Because the pK_a value of ethanol is

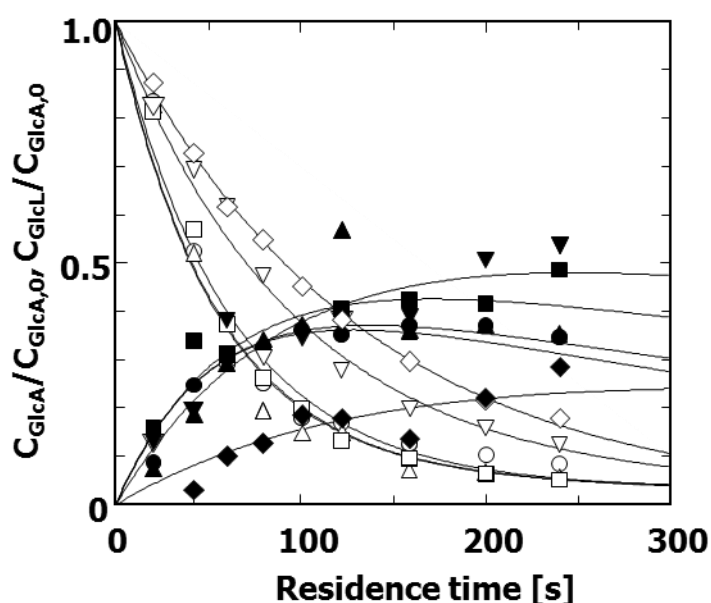


Figure 5-1. Disappearance of D-glucuronic acid (open symbols) and formation of D-glucuronolactone (closed symbols) for the treatment of 0.5% (w/w) D-glucuronic acid dissolved in aqueous solutions with different ethanol concentrations. Ethanol concentrations were 0% (water) (○, ●), 20% (w/w) (△, ▲), 40% (□, ■), 60% (▽, ▼), and 80% (◇, ◆). C_{GlcA} and C_{GlcL} represent D-glucuronic acid and D-glucuronolactone concentrations, respectively, and the subscript 0 indicates feed.

higher than that of water (Wyatt, 2014), the ion product of aqueous ethanol and dissociation of GlcA would change. As a result, the concentration of hydrogen ion would change. This effect may remarkably appear at 80% (v/v) ethanol for the suppression of GlcL formation. For other concentrations, the formation rate of GlcL did not significantly depend on the ethanol concentration, although the disappearance of GlcL seemed to be faster at lower ethanol concentrations at long residence times. This fact would be related to the suppression of GlcL at higher ethanol concentrations as described later.

Figure 5-2 shows the disappearance of GlcL and the formation of GlcA when 0.5% (w/w) GlcL was treated at 180°C in aqueous solutions with various ethanol concentrations. The disappearance of GlcL and the formation of GlcA were decelerated and suppressed, respectively, at higher ethanol concentrations. Comparison of the results shown in Fig. 5-2 to those shown in Fig. 5-1 revealed that the disappearance of GlcL was much slower than that of GlcA, and the formation of GlcA was very small when GlcL was treated in aqueous ethanol. These results indicate that the interconversion between GlcA and GlcL occurs reversibly; however, the conversion of GlcA to GlcL is preferred.

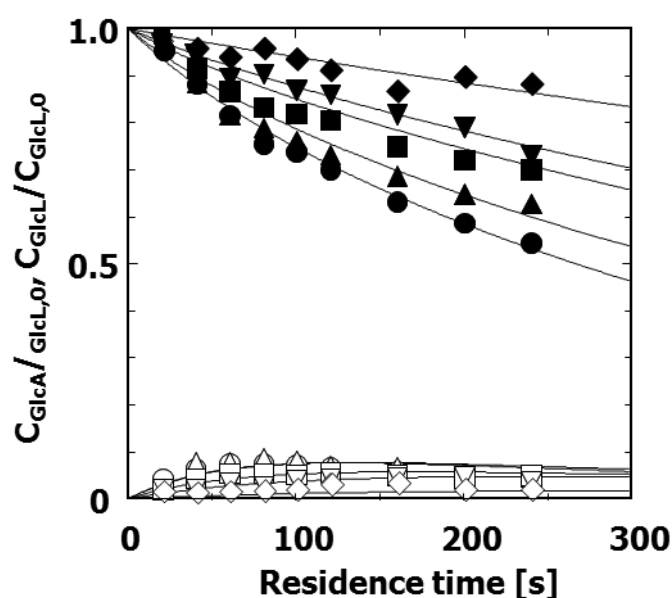


Figure 5-2. Disappearance of D-glucuronolactone (closed symbols) and formation of D-glucuronic acid (open symbols) for the treatment of 0.5% (w/w) D-glucuronolactone dissolved in aqueous solutions with different ethanol concentrations. The symbols and variables are the same as those in Fig. 5-1.

5.3.2 Kinetic analysis

Based on our previous study on the interconversion between GlcA and GlcL in subcritical water (Wang *et al.*, 2010), the results shown in Figs. 5-1 and 5-2 were kinetically analyzed. All of the reactions shown in Fig. 5-3 were assumed to obey first-order kinetics. The formation rates of GlcA and GlcL are formulated by Eqs. (5-1) and (5-2), respectively.

$$\frac{dC_A}{d\tau} = -(k_{AL} + k_{Ad})C_A + k_{LA}C_L \quad (5-1)$$

$$\frac{dC_L}{d\tau} = k_{AL}C_A - (k_{LA} + k_{Ld})C_L \quad (5-2)$$

where C is the concentration, τ is the residence time in the tubular reactor, and k is the first-order rate constant. The subscripts A and L represent GlcA and GlcL, respectively. The subscripts AL and LA indicate the conversion of GlcA to GlcL and the reverse conversion, respectively, and d represents degradation.

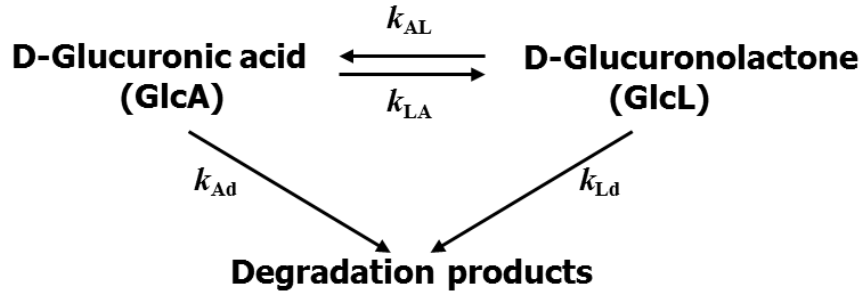


Figure 5-3. Assumed reaction scheme for the treatment of D-glucuronic acid or D-glucuronolactone in subcritical aqueous ethanol, and the definition of the rate constants.

The rate constants, k_{AL} , k_{LA} , k_{Ad} , and k_{Ld} , at each ethanol concentration were estimated to minimize the sum of the residual squares between the experimental and calculated values of C_A and C_L for both the reactions in Figs. 5-1 and 5-2 using the Solver of Microsoft Excel[®] 2010. The calculated concentrations were obtained by solving Eqs. (5-1) and (5-2) simultaneously by the numerical Euler method where the increment in τ was set to 1 s.

Figure 5-4 shows rate constants, k_{AL} , k_{LA} , k_{Ad} , and k_{Ld} , at various ethanol concentrations. The rate constants for the degradation of GlcA and GlcL, k_{Ad} and k_{Ld} , were smaller at higher

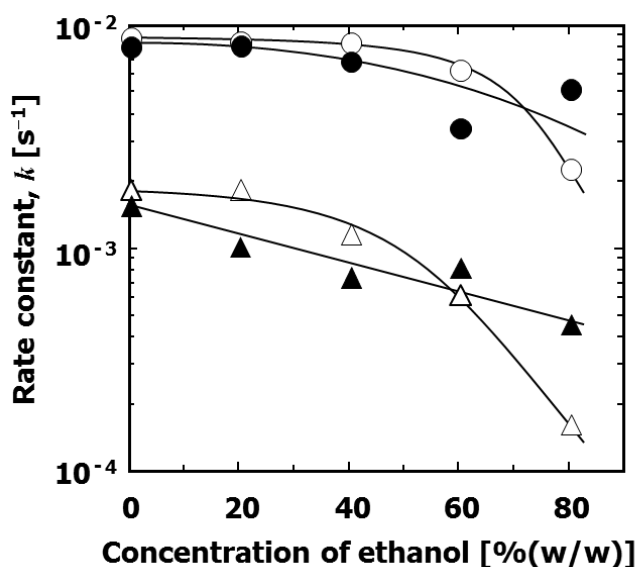


Figure 5-4. Dependencies of the rate constants for the conversion of D-glucuronic acid to D-glucuronolactone, k_{AL} (○), the reverse reaction, k_{LA} (△), and degradations of D-glucuronic acid to D-glucuronolactone, k_{Ad} (●) and k_{Ld} (▲), on the ethanol concentration at 180°C.

ethanol concentrations. In other words, the addition of ethanol into water suppressed the degradation of GlcA and GlcL. The rate constants for the interconversion between GlcA and GlcL, k_{AL} and k_{LA} , were also smaller at higher ethanol concentrations. The effect of ethanol on the rate constants became remarkably small at ethanol concentrations higher than 60% (w/w). Many factors affected the rate constants. All the rate constants reflect the concentration of hydrogen ion. The concentration of hydrogen ion would vary with the change in the ethanol concentration. Therefore, one of the reasons for the change in the rate constants would be related to the change in the ethanol concentration of the solvent. However, it remains unclear whether the higher ethanol concentration or the lower water concentration predominantly affects the smaller rate constants at higher ethanol concentrations.

5.3.3 Treatment at 200°C

GlcA, which was dissolved at 0.5% (w/w) in water, 40% (w/w) ethanol, or 80% (w/w) ethanol, was also treated at 200°C (Fig. 5-5). The disappearance of GlcA and the formation of GlcL were both slower at higher ethanol concentrations. This trend was similar to that at 180°C, although the degradation of GlcL was faster at 200°C than at 180°C. The 0.5% (w/w)

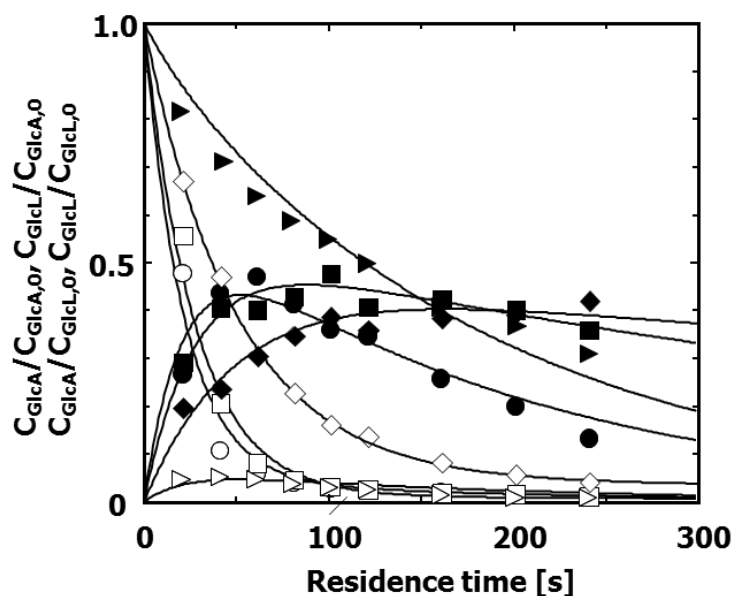


Figure 5-5. Treatment of D-glucuronic acid dissolved in water ($\circ$, $\bullet$), 40% ethanol ($\square$, $\blacksquare$), and 80% ethanol ($\diamond$, $\blacklozenge$), and treatment of D-glucuronolactone in water ($\triangleright$, $\blacktriangleright$) at 200°C. The open and closed symbols represent D-glucuronic acid and D-glucuronolactone, respectively.

GlcL dissolved in water was also treated at 200°C (Fig. 5-4). The formation of GlcA from GlcL was small at 200°C and was similar to that at 180°C.

5.4 Conclusion

The degradation and interconversion of GlcA and GlcL were kinetically analyzed under the assumptions of first-order kinetics in order to evaluate the rate constants for each process. The rate constants were smaller at higher ethanol concentrations. This was particularly manifested in the significantly low rate constants observed at ethanol concentrations higher than 60%.

Concluding Remarks

Chapter 1

The degradation of arabinose, xylose, ribose, and lyxose in subcritical water was measured at 200, 220, and 240°C. Ribose was the most rapidly degraded among the tested pentoses. The degradation of the glucuronic and galacturonic acids proceeded at lower temperatures than that of the pentoses, and was measured at 140, 150, and 160°C. The degradation processes of the pentoses and hexouronic acids could be expressed by the Weibull model, and the kinetic parameters were then estimated. The activation energy and frequency factor for the degradation of each substrate were estimated from the temperature dependence of the rate constant. The enthalpy-entropy compensation held for the degradation of the pentoses as well as the hexoses, which suggested that the degradation of the hexouronic acids proceeded through a mechanism different from that for pentoses and hexoses. The molar yield of a pentose to furfural was *ca.* 0.3 at any temperature irrespective of the pentose type. Acidic compounds were also formed from the pentoses in proportion to the amount of consumed substrates. The formation of acidic compounds resulted in a rapid decrease in pH.

Chapter 2

Mannose, fructose, and glucose were treated in subcritical water at 220°C, and the disappearance of each substrate and formation of other hexoses were observed at various residence times in a tubular reactor. Isomerization of mannose to fructose occurred most frequently, followed by mannose to glucose; no isomerization of glucose to mannose was observed. The temperature dependencies of mannose disappearance, the isomerization of mannose to fructose and glucose, and the resulting degradation of the produced fructose and glucose were observed in the range of 180 to 240°C. The activation energy and frequency factor for each process were evaluated according to the Arrhenius equation, and it was found that the enthalpy-entropy compensation for the processes was maintained.

Chapter 3

Linoleic acid was heated in the presence or absence of water in the temperature range of

200 to 260°C using a pressure-resistant batch reactor to examine the possibility of its conversion to conjugated isomers. The conversion occurred at very low yields, and the major products were the c9,t11 and t10,c12 conjugated linoleic acids. The ratio of the t10,c12 isomer to the c9,t11 one was about 0.6, and it did not depend on both the temperature and the ratio of linoleic acid to water. When a dilute potassium hydroxide was used as the aqueous solution, the yield of the conjugated isomers was lower than that of the isomers in the mixture of linoleic acid with distilled water.

Chapter 4

N-Acetyl-D-glucosamine (GlcNAc) was treated in subcritical fluids with ethanol contents of 0%–80% (w/w) using a tubular reactor at 190°C. The disappearance of GlcNAc in all the aqueous ethanol fluids obeyed first-order kinetics, and the rate constant was roughly proportional to the water molarity of the aqueous ethanol; this indicates that water plays an important role in the disappearance of GlcNAc. The pH of the reaction mixture decreased as the reaction proceeded and then plateaued because of the formation of a buffer system by glucosamine and the acetic acid liberated from GlcNAc. The ultraviolet absorption spectra of the reactor effluents suggested the formation of both carboxylic acids and furfurals. The formation of furfurals and color development were suppressed at higher ethanol contents.

Chapter 5

D-Glucuronic acid (GlcA) and D-glucuronolactone (GlcL) were treated with subcritical aqueous ethanol in the range of 0% to 80% (w/w) at 180°C in order to examine the effect of ethanol on the interconversion between GlcA and GlcL. When GlcA was treated at higher ethanol concentrations, less GlcA disappeared and more GlcL was formed compared to treatments at lower ethanol concentrations. For comparison, in the treatment of GlcL at higher ethanol concentrations, disappearance of GlcL was slower and less GlcA was formed. The degradation and interconversion of GlcA and GlcL were kinetically analyzed under the assumptions of first-order kinetics in order to evaluate the rate constants for each process. The rate constants were smaller at higher ethanol concentrations. This was particularly manifested

in the significantly low rate constants observed at ethanol concentrations higher than 60%. The treatments of GlcA and GlcL were also examined at 200°C, and the effect of ethanol at 200°C was similar to that at 180°C.

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Acknowledgment

I would like to express my sincere gratitude to my supervisor, Professor Shuji Adachi for his valuable guidance, suggestions, kind support, and for providing me this precious study opportunity in his laboratory.

I appreciate Professor Yukitaka Kimura and Assistant Professor Takashi Kobayashi for their elaborated guidance, considerable encouragements, and invaluable discussions.

I would like to express my gratitude to Professor Tamotsu Fujii, Professor Shota Tanimoto, and Professor Sumi Sugiyama for their sincere encouragements. I am very grateful to Ms. Natsumi Suzuki for her valuable cooperation in my experiment.

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