Carbohydrate Polymers 80 (2010) 296–302

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Depolymerization of sodium alginate under hydrothermal conditions

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ARTICLE INFO

Article history:
Received 4 October 2009
Received in revised form 13 November 2009
Accepted 18 November 2009
Available online 22 November 2009

Keywords:
Alginate
Mannuronic acid
Guluronic acid
Polysaccharide
Monosaccharide
Decomposition
Hydrolysis
Hydrothermal
Depolymerization

ABSTRACT

The depolymerization of sodium alginate with hydrothermal treatment (180–240 °C) was studied to understand the selectivity of monosaccharide generation. Alginate depolymerized to oligosaccharides, monosaccharides and decomposition products that included lactic acid and glycolic acid. Alginate depolymerization under hydrothermal conditions occurred in a manner that initially released mannuronic acid (M) and then was followed by the release of guluronic acid (G). Monosaccharides were generated through the hydrolysis of the glycosidic bonds between the unit monomer with different selectivities among M–M, M–G and G–G units rather than random sites within alginate. Hydrothermal treatments shows promise as a method to modify the structure of alginate polymers.

1. Introduction

Alginate is a linear hetero polysaccharide that consists of two-unit monosaccharides, D-mannuronic acid (M) and L-guluronic acid (G) that occur in homo polymeric M blocks, homo polymeric G blocks and hetero polymeric random MG blocks (Haug, Larsen, & Smidsrod, 1967b). The physical and chemical properties of alginate change with its molecular weight and M/G ratio that vary according to its origin (Gacesa, 1988; Haug, Myklesta, Larsen, & Smidsrod, 1967c). In science, some of the main uses of alginate is in tissue engineering (Augst, Kong, & Mooney, 2006), drug carrier studies (Tonnesen & Karlsen, 2002) and materials synthesis (Lu, Gao, & Komarneni, 2004). The design and tailoring of alginate polymers with the suitable molecular weight and monomer distribution is important in these applications (Draget, Smidsrod, & Skjak-Braek, 2002; Gacesa, 1988).

Present methods for depolymerizing alginate are enzymatic (Murata et al., 1993), acidic (Haug, Larsen, & Smidsrod, 1966; Haug et al., 1967b; Yang, Li, & Guan, 2004), alkali (Haug, Larsen, & Smidsrod, 1967a; Niemela & Sjostrom, 1985), and thermal treatment (Holme, Lindmo, Kristiansen, & Smidsrod, 2003). Enzymatic assays with alginate lyases, require long reaction times (ca. days) in which the 1–4 glycosidic linkages split in a β-elimination pathway, resulting in unsaturated non reducing terminals. Acid (Chandia, Matsuhiro, & Vasquez, 2001; Haug & Larsen, 1962; Haug et al., 1966) and base (Haug et al., 1967a; Niemela & Sjostrom, 1985) methods are fast, but require harsh chemicals.

Hydrothermal treatment, on the other hand, provides a method that is rapid for modifying the polymer properties without using harsh chemicals (Akiya & Savage, 2002; Brunner, 2009; Kruse, 2009). Hydrothermal treatment has been used for hydrolysis of cellulose (Hayashi, Fujita, Irie, Sasaki, & Shibata, 2004; Sasaki, Fang, Fukushima, Adschiri, & Arai, 2000), starch (Nagamori & Funazukuri, 2004), guar gum (Miyazawa & Funazukuri, 2006) and alginate (Holme, Davidsen, Kristiansen, & Smidsrod, 2008; Matsushima et al., 2005). In a previous study, Matsushima et al. (2005) studied the decomposition of alginate in subcritical water and supercritical water at temperatures from 250 to 375 °C with the goal to produce homopolymers. Their work showed that they could obtain a homopolymer containing 98% guluronic acid at 250 °C in 88 ms reaction time.

Since the reaction in supercritical water and subcritical water is very fast, the depolymerization sequence undoubtedly occurs at hydrothermal conditions and could allow the modification of alginate molecular weight distributions. In this work, the objective was to study the reaction of alginate under hydrothermal conditions (180–250 °C) and to develop a scheme for its depolymerization.

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doi:10.1016/j.carbpol.2009.11.032

2. Experimental

2.1. Materials and methods

2.1.1. Materials

Sodium alginate (300–400 mPa s) was obtained from Wako Pure Chemical Industries and was used as received. The characteristics of the sodium alginate, which were determined by gel permeation chromatography (GPC), differential thermal gravitical analyzer (TG) and ^{1}H NMR, are shown in Table 1.

Pullulan standard P-82 with known molecular weight, from 5.9 to 404 kDa, was obtained from Showa Denko and was used for constructing the calibration curve for the GPC analysis. Mannuronic and guluronic acid standards were prepared by procedures in the literature (Konno & Oba, 2007) and analysis with HPLC chromatography showed no other peaks. Distilled water was purified by a distillation apparatus (CPW-100, Advantec) and had a conductivity of 5.5 μS/m.

2.1.2. Analytical methods

High performance liquid chromatography (HPLC) was used in both qualitative and quantitative analyses of the monosaccharides, mannuronic acid and guluronic acid. Gel permeation chromatography (GPC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analyses were conducted to follow changes in the molecular weight distribution of the alginate. ^{1}H NMR analysis was conducted to evaluate the monomer sequence in the alginate polymer chain. Total organic carbon (TOC) analyses were used to check the carbon balances.

2.1.2.1. HPLC analysis. The HPLC analysis were used to quantify mannuronic acid and guluronic acid. The HPLC system was equipped with a refractive index (RI) detector (RI SE-61, SHODEX), two RSpak KC-811 columns (Showa Denko) and H_3PO_4 aqueous solution (5 mM) was used as mobile phase at a flow rate of 1.0 mL/min at 60 °C. The products mannuronic acid and guluronic acid were quantified according to the calibration curve constructed previously by the standards. Peaks corresponding to lactic acid and glycolic acid were not quantified due to their small amounts.

2.1.2.2. GPC analysis. The GPC system was equipped with an RI detector (RI-1530, JASCO), an auto sampler (AS-2055plus, JASCO) and column oven (CO-2065plus, JASCO). Asahipak GS-220HQ column and GS-520HQ column (Showa Denko) were used, and $NaNO_3$ aqueous solution (0.3 M) was used as the mobile phase at a flow rate of 1.0 mL/min at 60 °C. The calibration of the GPC was conducted by using pullulan P-82 standards.

2.1.2.3. MALDI-TOF-MS analysis. Direct measurement of molecular weights of the product compounds was confirmed with MALDI-TOF-MS. The MALDI-TOF-MS spectra were recorded with an AXIMA-CFR Plus (AXIMA-CFR Plus, Shimadzu) in the reflectron and the negative ion mode by collecting mass spectra with a nitrogen laser at 337 nm. Samples for the analyses were prepared by mixing 0.5 μL of the product solution with 0.5 μL of the matrix solution. The matrix solution was prepared by mixing a norharmane–acetonitrile solution and trifluoroacetic acid (0.1 M) at a volume ratio of 7:3. The norharmane–acetonitrile solution was prepared by mixing norharmane (10 mg) with acetonitrile (1 mL). Aliquots were placed on a stainless steel sample plate and then ionized by nitrogen laser pulses.

Table 1
Characterization of sodium alginate used in this work: weight average molecular weight (M_w), water content, mannuronic acid content (M), guluronic acid content (G) and M/G ratio.

M_w (Da)	Water content (%)	M%	G%	M/G ratio (−)
4.2×10^5	14	57	43	1.3

2.1.2.4. ^{1}H NMR analysis. The ratio between mannuronic acid and guluronic acid within the alginate was analyzed by an ^{1}H NMR technique given in the literature (Grasdalen, 1983; Grasdalen, Larsen, & Smidsrod, 1979). The ^{1}H NMR spectra were recorded with a 400 MHz spectrometer (Brunker Biospin DPX-400) at 80 °C and 80 scans. After the product was freeze-dried, the solid was dissolved in 0.6 mL of D_2O containing 0.1% acetonitrile as internal standard (σ = 2.06 for the methyl group).

The *G*% and *GG*% of the alginate were calculated by a literature method (Grasdalen, 1983; Grasdalen et al., 1979), and used three peak areas, *A*, *B*, *C*, as defined next. The area of *A* was defined as δ = 4.95∼5.17 (H-1 of guluronate), that of *B* was defined as δ = 4.55∼4.82 (H-5 of guluronate and H-1 of mannuronate) and that of *C* was defined as δ = 4.40∼4.50 (H-5 of guluronate), where *G*% and *GG*% were calculated by the following equations:

$$G\%[\%] = \frac{\text{area of } A}{(\text{area of } B) + (\text{area of } C)} \times 100 \quad (1)$$

$$GG\%[\%] = \frac{\text{area of } C}{(\text{area of } B) + (\text{area of } C)} \times 100 \quad (2)$$

2.1.2.5. TOC analysis. The total carbon concentration of the liquid product was analyzed by TOC (Shimadzu, model TOC-5000A) and the carbon recovery of the liquid phase was evaluated. The carbon balances of the experiments conducted at 180 °C were approximately 96% for reaction times of 6 min and 59% at 14 min, 50% at 30 min. The decrease of carbon recovery indicated that hydrothermal treatment produced carbon products that were not soluble in the liquid phase. It was observed that both solid and gas products formed especially at the longer reaction times.

2.1.3. Procedure

An aqueous solution of sodium alginate with a concentration of 2.0 wt.% was prepared prior to the experiments. The alginate solution (3.0 g) was loaded into stainless steel tube (SUS316) batch reactors that had an inner volume of 6.0 cm^3. Before heating, each reactor was purged with Ar gas to removal air and then sealed. Heating of the reactor was initiated by immersion of the reactor into a molten salt bath that was controlled at the desired reaction temperature to within ±3 °C. The reaction temperatures chosen for study were 180, 200, 220 and 240 °C. The reaction was terminated by transferring the reactor from the molten salt bath to a water bath that was at room temperature. The reaction time was defined as the time when the reactor was first placed into the molten salt bath until the reactor was quenched in the water bath. The temperature profile in the reactor was measured by using a batch reactor having a thermocouple placed in the liquid phase of the solution. The time required for the reactor to reach the reaction temperature (200 °C) was 2 min, while the time for cooling to below 100 °C was 30 s. Experiments conducted at 2 min reaction time, therefore, have higher uncertainty in the results. After the reactor was cool, its content was washed with distilled water and the effluent analyzed. At least three experiments were conducted for each reaction condition. The average and the deviation of the yields are shown as data points and error bars in the graphs.

3. Results and discussion

3.1. General characteristics of hydrothermal treated samples

Reaction of a 2 wt.% solution of alginate at 180 °C for 6, 14 and 30 min gave product solutions that became progressively darker (Figs. S1 and SI) which indicated rapid reaction of alginate. Some general characteristics of the reacted solutions are noted here before discussing the results in detail. HPLC chromatograms of the product solutions had identifiable peaks with a retention time corresponding to glycolic acid and lactic acid and also unidentifiable peaks (Figs. S2 and SI). It was observed qualitatively that the reacted solutions had viscosities that were progressively lower according to the reaction time, which suggests that the hydrothermal treatment caused the molecular weight of the alginate to decrease. The decrease in molecular weight is discussed later with the obtained GPC chromatograms. The pH of the raw material in solution was 7.1. After the reaction, the pH of the solutions were 4.8, 4.7 and 4.9 for 6, 14 and 30 min hydrothermal treated samples, respectively. The decrease in pH for hydrothermal treated solutions confirmed the formation of water soluble acids. In the examination of the reactor contents, gases were formed as well as solids, especially at the larger reaction times (ca. 14 min), however, these were not quantified.

3.2. Generation of mannuronic acid and guluronic acid

Fig. 1 shows results for the combined yield of monosaccharides, mannuronic and guluronic acid, as a function of reaction time over the range of temperatures studied. The combined monosaccharide yield as given by the peak in the data at each temperature tended to increase and then decrease as reaction time progressed, which is evidence that the initial monosaccharides formed subsequently decomposed. The highest combined monosaccharide yield for each temperature was shifted to shorter reaction times as the reaction temperature became higher, which is similar to previous observations for monomeric species obtained from hydrothermal treatment of cellulose (Hayashi et al., 2004), starch (Nagamori & Funazukuri, 2004) and guar gum (Miyazawa & Funazukuri, 2006). The highest combined monosaccharide yield of alginate is much lower than that which can be obtained for other polysaccharides (Hayashi et al., 2004; Miyazawa & Funazukuri, 2006; Nagamori & Funazukuri, 2004). Alginate monosaccharides, mannuronic and guluronic acid, are much more reactive than cellulose and its oligomers. The lower decomposition temperatures of alginate compared with cellulose, starch or guar gum, can be explained by its high hydrophilia and also its self-catalytic nature due to the carboxylic acid in its chemical structure (Smidsrod, Haug, & Larsen, 1966) that promotes hydrolysis earlier than when polysaccharides are treated under hydrothermal conditions. The conditions for dissolution of polysaccharides has noted to be important in hydrothermal decomposition reaction of cellulose (Ogihara, Smith, Inomata, & Arai, 2005).

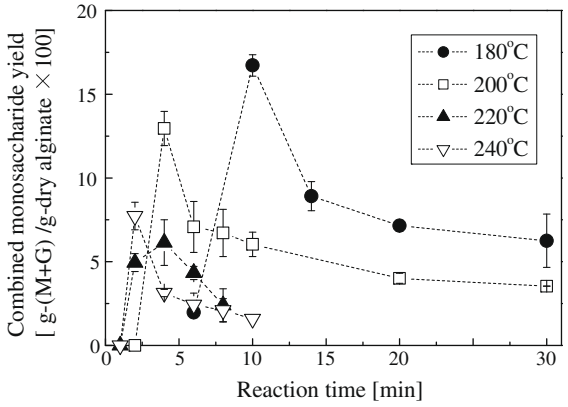


Fig. 1. Combined yield of mannuronic and guluronic acid as a function of reaction time obtained from treating alginate under hydrothermal conditions at 180, 200, 220 and 240 °C.

Fig. 2(a–d) shows the individual yields of mannuronic acid and guluronic acid as a function of reaction time for each temperature. Mannuronic acid gave higher yields than guluronic acid at low reaction times, but its yield decreased to a value below that of guluronic acid as reaction time increased. This means that the decomposition of alginate under hydrothermal conditions probably occurred in a manner in which mannuronic acid was initially released and was severed at the glycosidic bonds between mannuronic–mannuronic (M–M) groups. The results show the possibility for selective recovery of monosaccharides with hydrothermal treatment.

3.3. Alginate decomposition pathway

In the previous section, it was shown that alginate monosaccharides, mannuronic and guluronic acid, exhibited different reaction times for peak (maximum) yields and also that those compounds had different reactivities under hydrothermal conditions. GPC, MALDI-TOF-MS and ^{1}H NMR analyses were conducted to study the decomposition pathway of alginate. The reaction products at 180 °C were chosen to study in detail, since monosaccharides yield were high and alginate decomposition were slow enough to follow.

Fig. 3 shows a GPC chromatogram of alginate treated under hydrothermal conditions for various reaction times. The raw alginate solution had a weight average molecular weight of 4.2×10^5 Da. Product molecular weight distribution shifted towards lower values as reaction time increased. At 6 min (Fig. 3), alginate decomposed into monosaccharides as designated by the arrow that marks the monosaccharide peak (mannuronic acid and guluronic acid). From the GPC results (Fig. 3), it can be seen that compounds having molecular weights that were lower than the monosaccharides were formed. As the reaction time increased to 10 min (Fig. 3), the molecular weight distribution shifted towards smaller values, which indicates an increase in monosaccharides and decomposition products. At 20 min reaction time (Fig. 3), the intensity of the monosaccharide peak decreased compared with results for 10 min reaction time and the peak representing the decomposition products increased. Acids and low molecular weight products were most likely formed through the decomposition of the monosaccharides, however, they could have also been formed as alginate undergoes depolymerization. In an attempt to distinguish between these possible pathways, MALDI-TOF-MS of the product solutions was measured as discussed next.

Fig. 4 shows a representative MALDI-TOF-MS chart of the product solution obtained from treating alginate under hydrothermal conditions at 180 °C for 10 min. High intensity readings were observed at 723, 795, 898, 1072, 1246, 1418 m/z where 795 m/z is the peak for the matrix used (Fig. 4). These results show that oligosaccharides were produced during the hydrothermal treatment of alginate. The molecular weight of the unit monosaccharides of alginate (mannuronic acid or guluronic acid) is 176, which was practically equal to the mass difference observed between neighboring peaks in the spectra. The consistent differences between the peaks are evidence that the decomposition of alginate under hydrothermal conditions proceeds through the hydrolysis of the glycosidic bonds between the unit monomer and not through random sites within alginate. If random decomposition of the alginate occurred, the MALDI-TOF-MS spectrum should give a broad molecular distri-

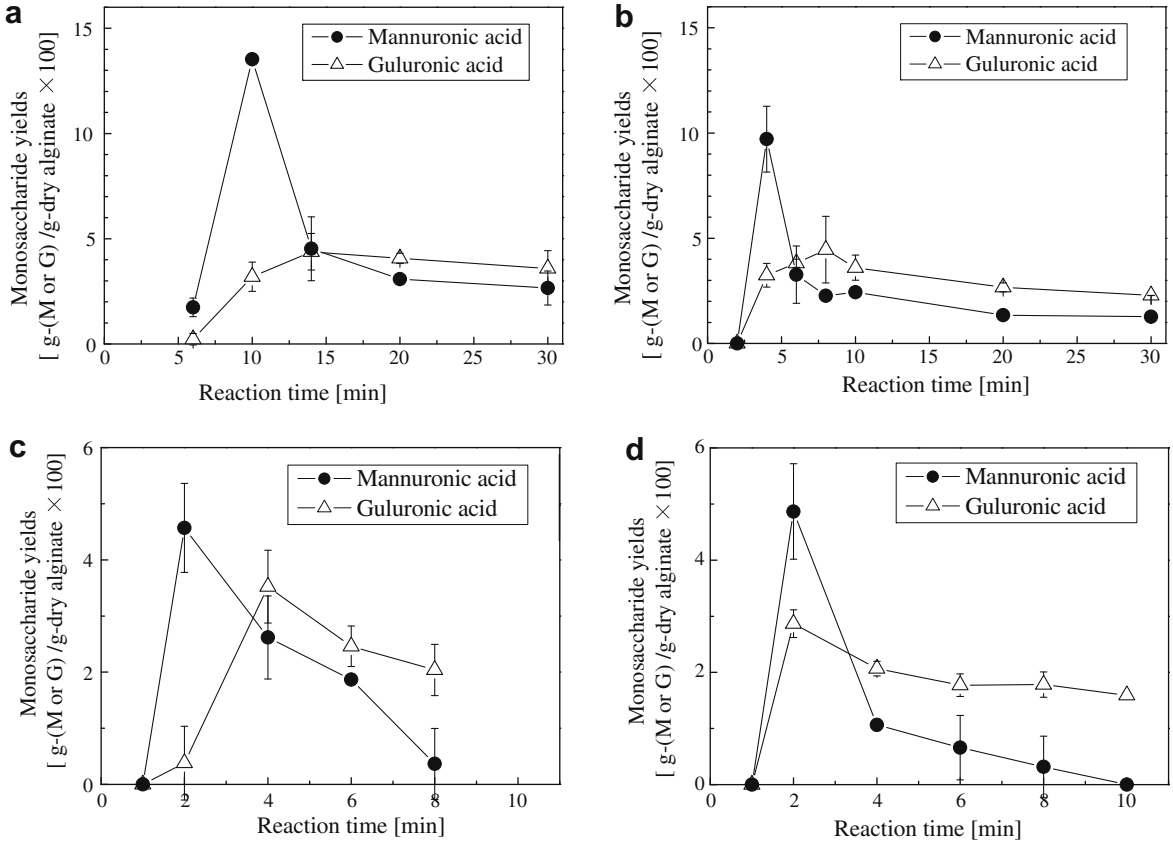


Fig. 2. Yield of mannuronic and guluronic acid as a function of reaction time obtained from treating alginate under hydrothermal conditions at (a) 180 °C, (b) 200 °C, (c) 220 °C and (d) 240 °C.

bution that does not have a characteristic difference of 176 between the neighboring peaks. Therefore, the decomposition products that were observed at 180 °C for 6 min in Fig. 3 were most likely generated from monosaccharides rather than from alginate directly. Under hydrothermal conditions, it can be concluded that the decomposition of monosaccharides to small molecular weight compounds readily occurs.

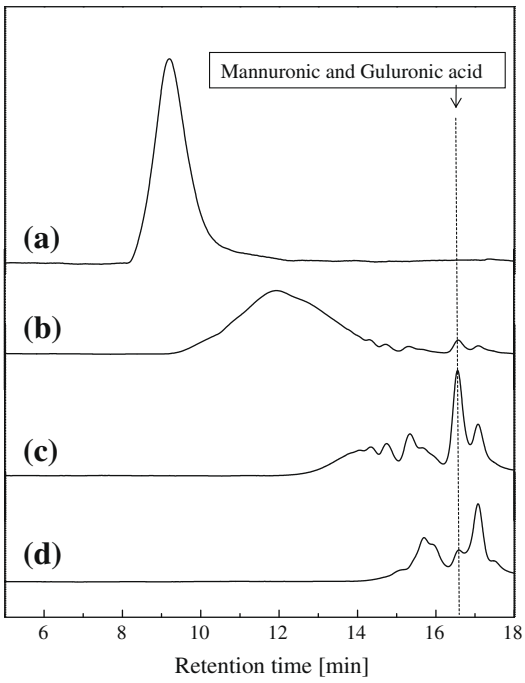


Fig. 3. Gel permeation chromatograms of product solutions obtained from treatment of a 2 wt.% alginate solution under hydrothermal conditions at 180 °C with RI detector. (Reaction times: (a) raw material, (b) 6 min, (c) 10 min and (d) 20 min).

The decomposition of alginate under hydrothermal conditions seems to occur through an acid hydrolysis pathway and not through the base hydrolysis β-elimination pathway. In a previous study (Haug, Smidsrod, & Larsen, 1963) it was reported the rate of base hydrolysis of alginate is significantly lower than the acid hydrolysis at conditions of pH below 5. We acknowledge that the pH of the hydrothermal treated samples were in the range of 4.7–4.9.

In Fig. 2, it was shown that mannuronic acid was initially formed and then guluronic acid was subsequently formed for all of the temperatures that were studied. This indicates that a selective hydrolysis occurred between the four types of glycosidic bonds (M–M, M–G, G–M and G–G) in some sequence as depolymerization proceeded.

To understand the hydrolysis pathway between these different glycosidic bonds, we conducted ^{1}H NMR analysis and evaluated the monomer sequence in the alginate polymer chain. Fig. 5 shows a typical ^{1}H NMR spectrum of alginate solution that was treated under hydrothermal conditions. Table 2 shows the monomer composition and sequence of the alginate before and after hydrothermal treatment. The monomer composition in the alginate decreased for mannuronic acid and increased for guluronic acid after hydrothermal treatment. Selective hydrolysis of mannuronic acid seem to occur, which is consistent with the HPLC analyses in Fig. 2. The results also showed that as the GG sequence increased, MM decreased and MG did not show any change, which indicates that selective hydrolysis occurs at the M–M glycosidic bonds rather than at the G–G glycosidic bonds.

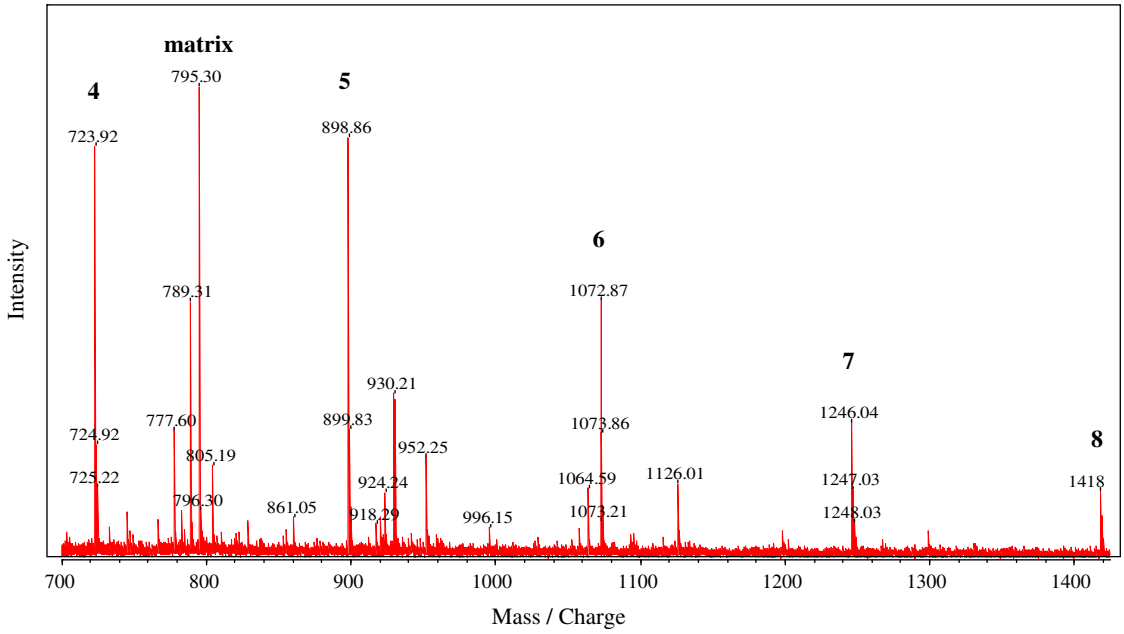


Fig. 4. Number of monosaccharide units of product (bold numerals) after treatment under hydrothermal conditions determined from MALDI-TOF-MS. Conditions: 2 wt.% alginate concentration, hydrothermal treatment at 180 °C and 6 min reaction.

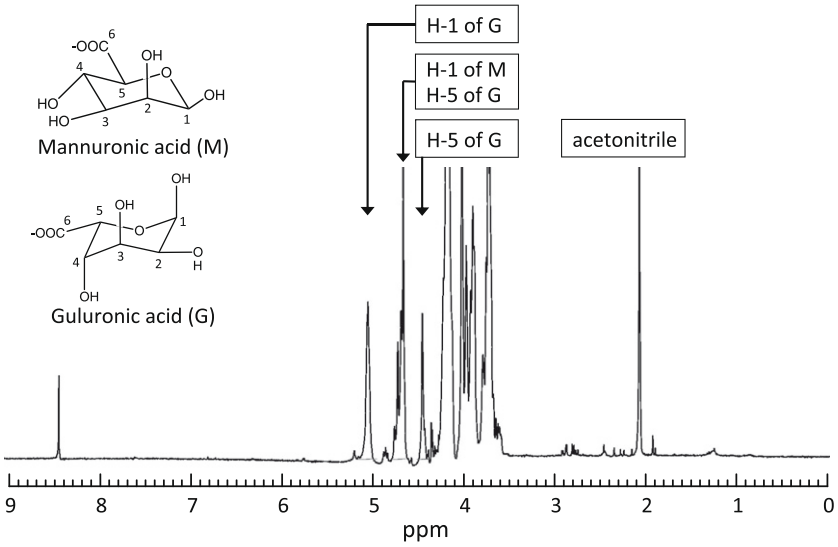


Fig. 5. ^{1}H NMR spectrum of a 2 wt.% alginate solution treated under hydrothermal conditions (180 °C and 6 min reaction time).

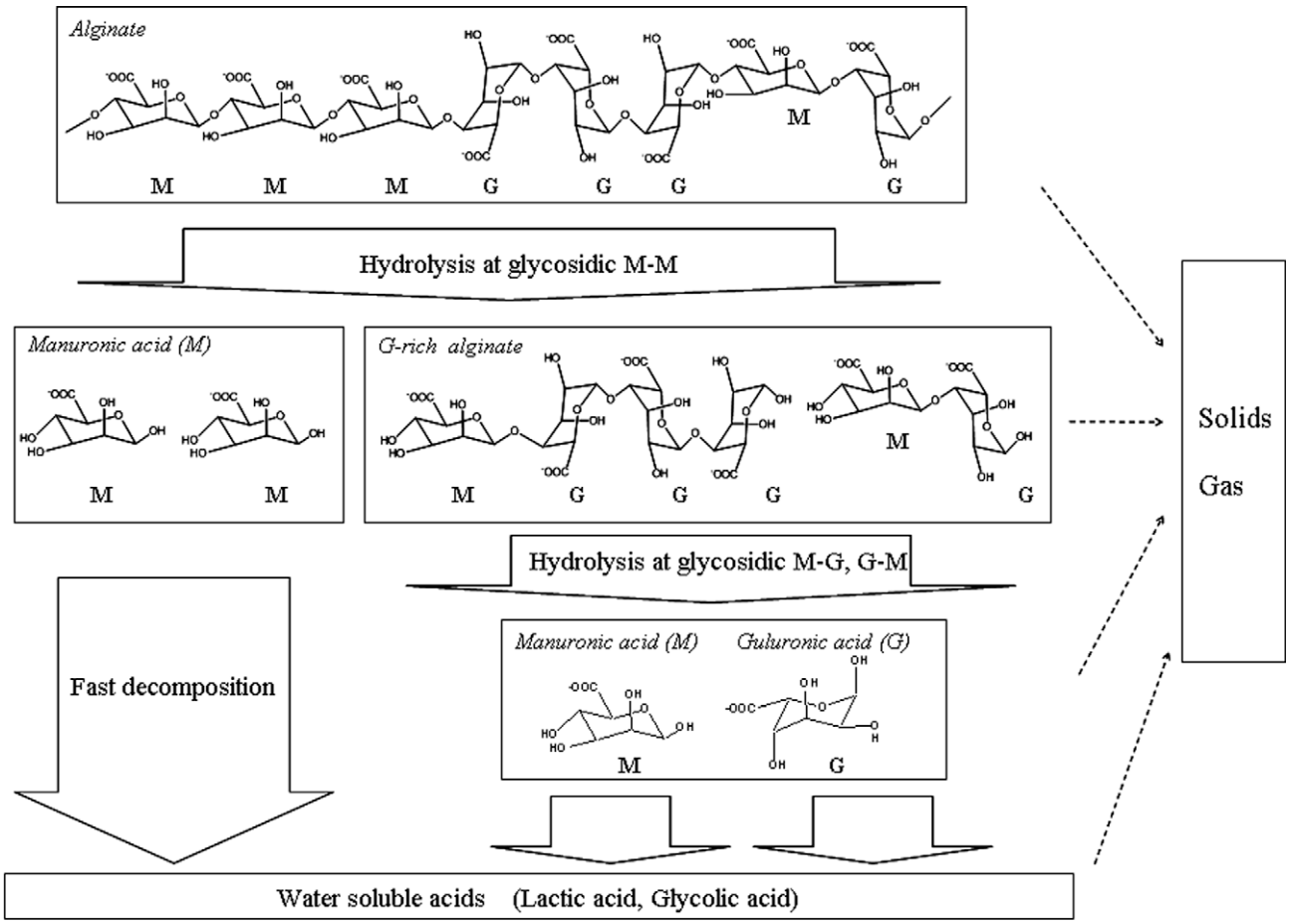
Table 2
Mannuronic acid (M) and guluronic acid (G) composition of sodium alginate before and after hydrothermal treatment according to ^{1}H NMR analysis. Conditions: 2 wt.% sodium alginate in water and reaction temperature of 180 °C.

	M%	G%	GG%	MG%	MM%
Raw material	57	43	18	25	32
6 min	51	49	24	25	26
10 min	17	83	42	n.p.	n.p.

n.p., not present.

Previous studies on decomposition of alginate under acidic conditions suggest that the selective hydrolysis occur at the MG glycosidic bonds (Haug & Larsen, 1963). These studies have been conducted at lower temperatures (100 °C) than used in this work. According to the results in this work, increasing the reaction temperature changed the order of the kinetic rates of the hydrolysis of these three (M–G, M–M, G–G) glycosidic bonds. A study on the decomposition kinetics of alginate using a flow apparatus, having better control of reaction time and temperatures, might provide better understanding to these kinetic effects.

Based on the results obtained in this work, a reaction pathway can be proposed for the decomposition of alginate under hydrothermal conditions (Scheme 1). In the proposed pathway, the decomposition of alginate occurs by hydrolysis at the glycosidic bonds with different selectivities. We consider that this is a result from the difference in the kinetics of hydrolysis of the three types of glycosidic bonds in the order of M–M, M–G and G–G. Initially mannuronic acid is produced from alginate and then guluronic acid forms from a G-rich alginate oligomer. After mannuronic acid forms, it decomposed rapidly to water soluble acids, solids and gas.



Scheme 1. Depolymerization pathway of sodium alginate under hydrothermal conditions.

4. Conclusions

The reaction of alginate under hydrothermal conditions was studied. Alginate depolymerizes under hydrothermal conditions to monosaccharides, in a manner that initially releases mannuronic acid and then is followed by the release of guluronic acid. The decomposition reaction of alginate is promoted by increasing temperature, however, the monosaccharide yields decrease with increasing temperature. As the reaction proceeds, monosaccharide decomposition occurs. The decomposition of alginate occurs by hydrolysis at the glycosidic bonds, however, different selectivities exist between M–M, M–G and G–G units. Selective production of MM, MG or GG oligomers or of monosaccharides, mannuronic acid and guluronic acid, may be possible by using the kinetic differences of hydrolysis with hydrothermal treatment. Experiments are in progress on studying the molecular weight modification with a flow apparatus and will be reported in the future.

Acknowledgments

We thank Dr. Masao Konno, Riken Shokuhin Co. Ltd. (Japan, Miyagi), for providing the monosaccharides standards. We thank Mr. Hiroaki Saito (Tohoku University, Japan) for the NMR analysis, Ms. Aya Haneda (Tohoku University, Japan) for the MALDI-TOF-MS analysis. Financial support from Global COE (Tohoku University) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2009.11.032.

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