Non-catalytic Conversion of Chitin into Chromogen I in High-temperature Water

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Abstract

The non-catalytic conversion of chitin into *N*-acetyl-D-glucosamine (GlcNAc) derivatives such as 2-acetamido-2,3-dideoxy-D-erythro-hex-2-enofuranose (Chromogen I) was investigated in high-temperature water at 290–390 °C and 25 MPa with a reaction time of 0–180 min. High-temperature water treatment is a promising method for chitin conversion as it does not require the use of any additional organic solvents or ionic liquids. A semi-batch reactor was developed to control the reaction temperature and time. It was found that the chitin powder could be converted into a water-soluble fraction in ~90% yield, with Chromogen I being obtained in a maximum yield of 2.6%. Furthermore, a kinetic model was developed to estimate the reaction rate for the conversion of the chitin powder to the water-soluble fraction.

Keywords: chitin, glucosamine, supercritical water, hydrothermal treatment

1. Introduction

Chitin is the second most abundant biomass on earth, and is a major component in the cell walls of fungi as well as in the exoskeletons of insects and crustaceans [1]. *N*-Acetyl-D-glucosamine (GlcNAc) is an important unit in the chitin polysaccharide, and GlcNAc derivatives such as 2-acetamido-2,3-dideoxy-D-erythro-hex-2-enofuranose (Chromogen I), 3-acetamido-5-(1',2'-dihydroxyethyl)furan (Chromogen III), and 3-acetamido-5-acetylfuran (3A5AF) can be obtained from this species upon dehydration (Figure 1) [2]. These GlcNAc derivatives exhibit potent biological activities and so have recently attracted attention as new functional food additives and medicines [2–9].

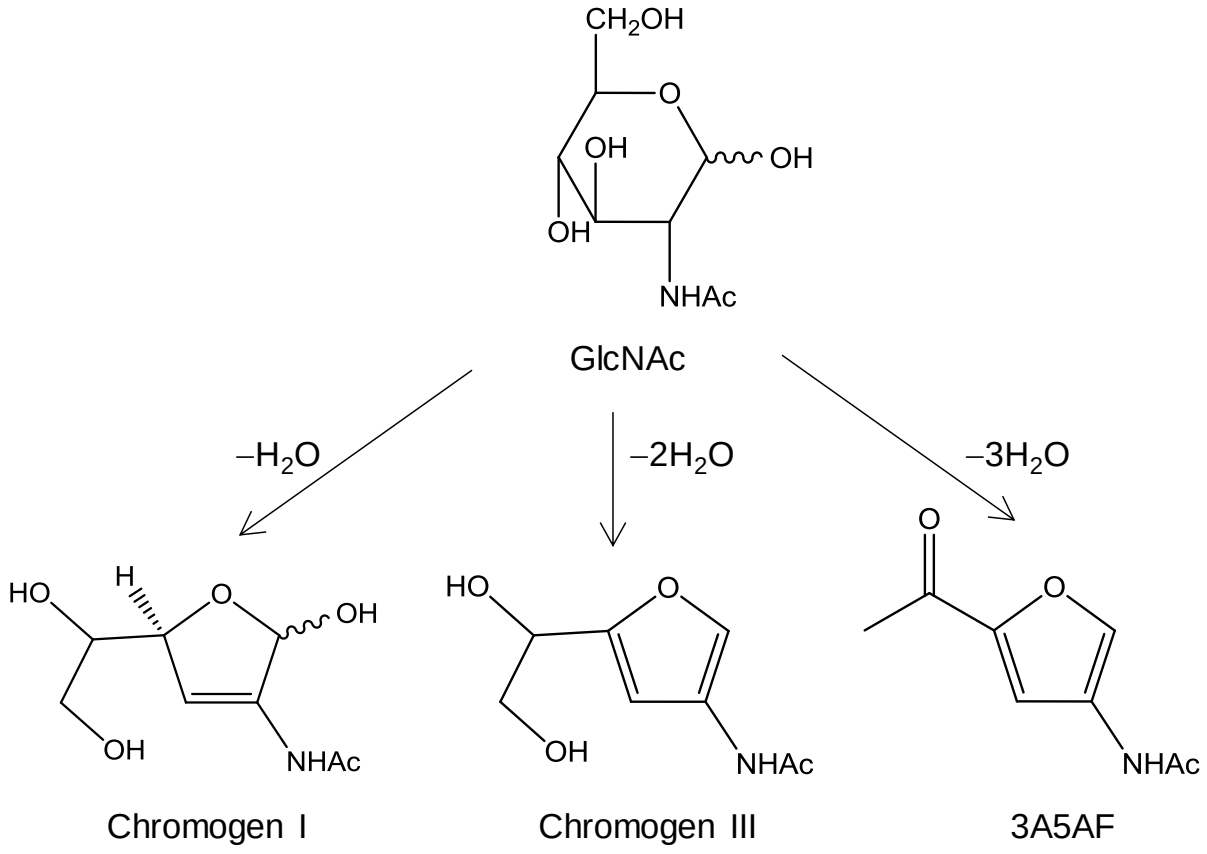

Fig. 1 Example GlcNAc derivatives obtained from chitin in high-temperature water.

To date, organic solvents and ionic liquids have generally been employed in the preparation of GlcNAc derivatives from chitin [10–15]. For example, Chen et al. reported that the most effective system for the preparation of such derivatives was based on the use of an ionic liquid, namely 1-butyl-3-methylimidazolium chloride, as the solvent with boric acid and HCl as additives, resulting in a 28.5% yield of 3A5AF at 180 °C [14]. As such, the development of a sustainable, green, and eco-friendly method for the conversion of chitin without the requirement for organic solvents or ionic liquids is desirable.

In this context, high-temperature water has been recognized as a green chemical medium for some organic reactions because such reactions can proceed without the use of a catalyst [16–18]. Indeed, we have reported the non-catalytic dehydration of GlcNAc in high-temperature water at 120–220 °C and 25 MPa, where a reaction time of 7–39 s affords Chromogen I and III [19]. In addition, it has been reported that the hydrolysis of chitin proceeds in high-temperature water above 300 °C [20–25]. During this hydrolysis process, the molecular weight of chitin decreased and water-soluble low-molecular weight chitin chains were formed [26]; however, GlcNAc was not obtained due to immediate dehydration of the low-molecular weight chitin chains at the reducing end [27]. These results therefore suggest that the dehydrated GlcNAc derivatives could be obtained not only from GlcNAc but also from chitin.

Thus, we herein report our investigation into the production of GlcNAc derivatives such as Chromogen I, III, and 3A5AF from chitin in high-temperature water between 290 and 390 °C. A semi-batch type apparatus will be employed for this purpose. As the optimal temperatures for GlcNAc dehydration and chitin hydrolysis differ, we consider that it may be necessary to remove the obtained products from the high-temperature reactor immediately upon their production, and also to subsequently cool these products rapidly to inhibit any further decomposition. Furthermore, we develop a kinetic model to estimate the reaction rate for the conversion of the chitin powder to the water-soluble fraction.

2. Experimental

Crab shell chitin was obtained from Yaegaki Bio-industry, Inc. (Himeji, Japan). High-performance liquid chromatography (HPLC) grade acetonitrile was purchased from Thermo Fisher Scientific, Inc (Waltham, MA, USA). All chemicals were employed without further purification. Distilled water was obtained from a water distillation apparatus (Model WG-220, Yamato Scientific Co., Tokyo, Japan). Chromogen I, Chromogen III, and 3A5AF were synthesized according to previously reported methods [2,3,19] and were used as the standard samples for HPLC analysis.

High-temperature water treatment of the chitin powder was carried out using a semi-batch apparatus (Fig. 2), which consisted of a water loading unit, a water preheating

unit, a reactor loaded with chitin, a heat exchanger, a pressure control unit, and a solution recovery unit. For the high-temperature water treatment process, a sample of the chitin powder (0.2 g) was placed in the reactor, and a stainless-steel sintered filter was fitted at the end of the reactor. Distilled water was then introduced into the by-pass line and the reactor at a flow rate of 3 mL min^{-1} using HPLC pumps to pressurize the system to 25 MPa. This pressure was maintained using a back-pressure regulator. Initially, the 3-way valve was set to by-pass and the high-temperature water flowed only through the by-pass line. After the temperature of the by-pass line (as monitored by the T2 thermocouple) reached the desired temperature, the 3-way valve was switched to the reactor side and the high-temperature water was introduced into the reactor. This switching of the 3-way valve was defined as $t = 0$ in terms of the reaction time. The temperature inside the reactor was monitored using the T3 thermocouple, which was inserted directly into the reactor. The water-soluble products were collected at the outlet of the reactor by rapid quenching with a cooling jacket and continuous transfer to sampling bottles. After cooling, the solid residue was recovered; however, the majority of the solid residue remained in the pores of the stainless-steel sintered filter. As such, the mass of the solid residue was measured using the difference of the filter weight before and after treatment. Since recovery of the solid residue from the pores of the stainless-steel sintered filter is difficult, analysis of the solid residue was not possible.

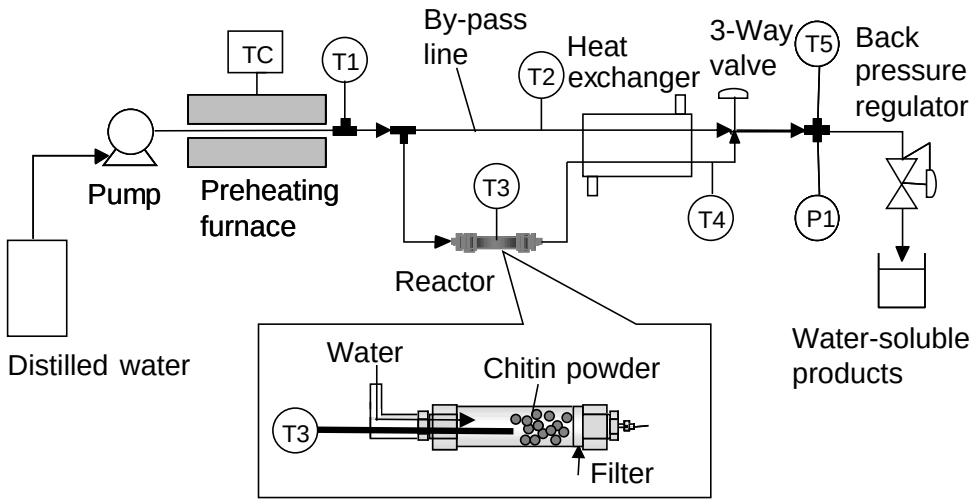

Fig. 2 The semi-batch reactor employed herein for chitin conversion in high-temperature water.

The quantity of organic carbon in the water-soluble fractions was evaluated using a total organic carbon analyzer (Model TOC-VCPN, Shimadzu Corp., Kyoto, Japan). HPLC analysis was carried out using a Shimadzu Intelligent system liquid chromatograph and detection at 210 nm. For Chromogen I, a Unison UK-Amino column (4.6 × 250 mm, Imtakt Corp., Kyoto, Japan) was used at 40 °C with 95% CH_3CN as the solvent at a flow rate of 1.0 mL min^{-1}. For Chromogen III and 3A5AF, a Unison US-C18 column (4.6 × 250 mm, Imtakt Corp., Kyoto, Japan) was used at 40 °C with 10% CH_3CN as the solvent at a flow rate of 1.0 mL min^{-1}.

The solid residue yield, water-soluble carbon yield, and the product yield are defined as given below:

Solid residue yield (wt%) = Mass of solid residue (g)/Mass of chitin loaded (g) × 100 (1)

Water-soluble carbon yield (%) =

Moles of carbon in the aqueous solution/Moles of carbon in chitin loaded × 100 (2)

Product yield (%) =

Moles of product/Moles of GlcNAc units in chitin loaded × 100 (3)

3. Results and discussion

Table 1 provides a summary of the experimental conditions employed and the results obtained based upon the material recovered from the semi-batch reactor. As described below, the reaction times of the various runs ranged from 30 to 180 min. The experiments at 380, 385, and 390 °C were performed to check the reproducibility, although the conditions were not exactly identical. Therefore, there is a slight variation (a few percent) in the experimental results. Although the original solid chitin powder is not water-soluble, almost all of this powder was converted into water-soluble fractions following high-temperature water treatment between 290 and 390 °C, with the main product being Chromogen I (<3% yield). No traces of GlcNAc were observed. In addition, small peaks corresponding to Chromogen III and 3A5AF were observed by HPLC analysis; however, their quantitative analysis was difficult, and so their yields were quoted as <0.5% (see Table 1). Indeed, the sum of the identified products was <4%, thereby indicating that ~90% of the products were unknown. Indeed, HPLC analysis showed many weak signals corresponding to the unknown products, which indicated that chitin was converted into various products and not only GlcNAc derivatives.

Table 1 Effect of high-temperature water treatment on the product yields from the semi-batch reactor

Run	Final Temperature [°C]	Solid Residue Yield [wt%]	Water-soluble Carbon Yield [%]	Product yield [%]			
				GlcNAc	Chromogen I	Choromgen III	3A5AF
1	290	4.0	89.6	0	0.7	<0.5	<0.5
2	360	1.5	86.9	0	2.6	<0.5	<0.5
3	380	4.3	93.2	0	1.6	<0.5	<0.5
4	385	0.8	90.2	0	1.4	<0.5	<0.5
5	390	0.4	86.7	0	0.6	<0.5	<0.5

No reports currently exist into the synthesis of Chromogen I from chitin powder in the presence or absence of a catalyst or in either an organic solvent or an ionic liquid. In addition, only a few reports exist into on the synthesis of 3A5AF from chitin powder using a catalyst either in an organic solvent or an ionic liquid [14]. As such, this study demonstrates the formation of Chromogen I from chitin powder in both types of reaction medium for the first time.

The merit of high-temperature water treatment is the dehydration of a single water molecule from chitin powder due to the low catalytic activity of high-temperature water. In contrast, where a catalyst is employed either in an organic solvent or an ionic liquid, the high catalytic activity promotes the dehydration of three water molecules to give 3A5AF rather than Chromogen I.

Although analysis of the solid residue could not be conducted, we previously reported that prolonged treatment in high-temperature water changed the chemical structure of chitin, as confirmed by thermogravimetric analysis and Fourier transform infrared spectroscopy [22, 23, 26]. It was therefore assumed that the solid residue did not maintain the chemical structure of the original chitin. As the main identified product was Chromogen I, we chose to focus on the yield of this compound.

Figure 3 shows the temporal variations in temperature monitored by the T3 thermocouples, in addition to the water-soluble carbon yield, and the Chromogen I yield of Run 1. As indicated, the temperature increased when the 3-way valve was switched at $t = 0$, reaching 290 °C after ~25 min. The water-soluble carbon yield peaked at 24% after 23 min prior to decreasing with longer treatment times. The sum of the water-soluble carbon yield obtained between 0 and 180 min was 89.6%, as shown in Table 1. The Chromogen I yield also peaked at ~23 min with a value of 0.18%, again prior to decreasing gradually upon increasing the treatment time. The sum of the Chromogen I yield obtained between 0 and 180 min was 0.7%.

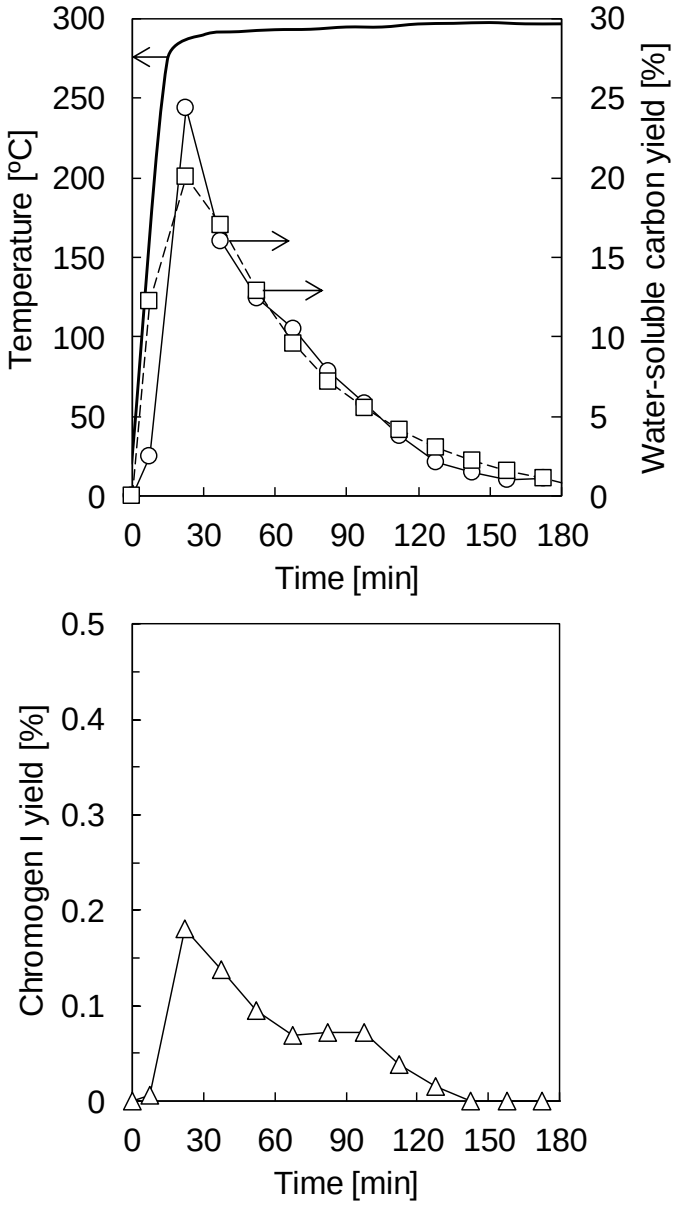

Fig. 3 Results of the chitin conversion reaction in high-temperature water (Run 1). The water-soluble carbon yield: (○) experimental and (□) calculated results.

Figure 4 shows the results of Run 2, where the temperature reached 360 °C after ~10 min. In this case, the water-soluble carbon yield peaked at 28% after ~17 min and then decreased quickly compared with Run 1. The sum of the water-soluble carbon yields obtained

over the initial 60 min was 86.9%. In addition, the Chromogen I yield peaked at 0.95% after ~17 min prior to decreasing rapidly (again compared with Run 1). The sum of the Chromogen I yields between 0 and 60 min was 2.6%.

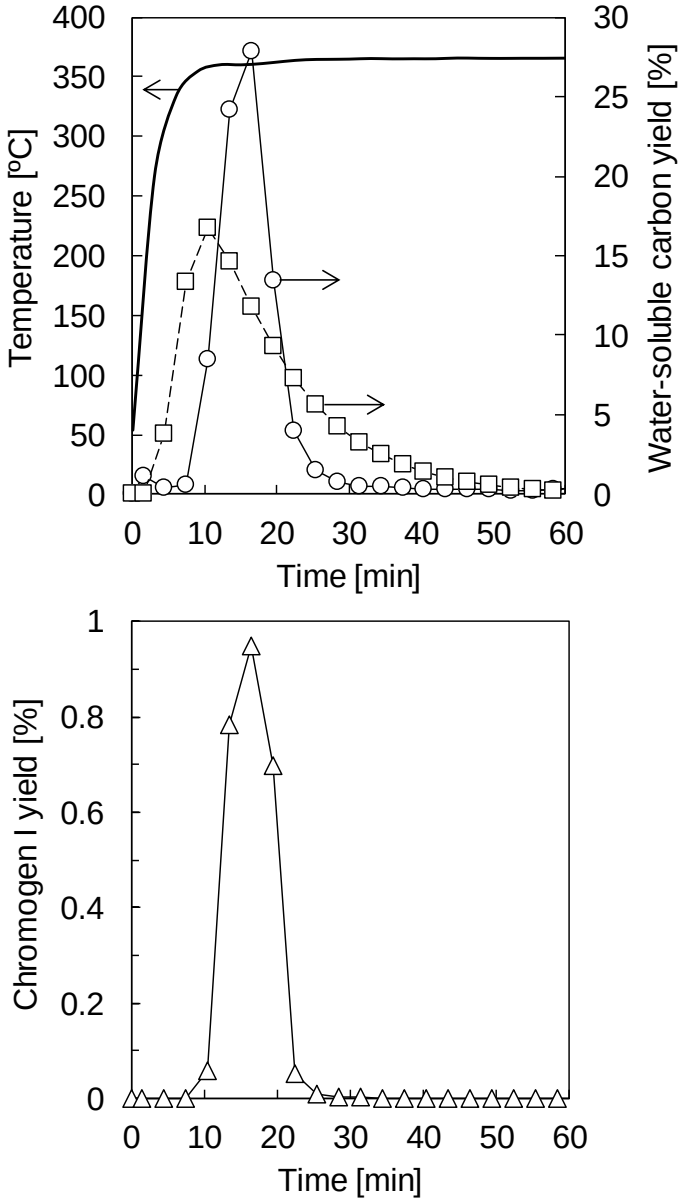

Fig. 4 Results of the chitin conversion reaction in high-temperature water (Run 2). The water-soluble carbon yield: (○) experimental and (□) calculated results.

As shown in Figure 5 (Run 3), the temperature reached 380 °C after ~10 min. In this case, the water-soluble carbon yield peaked at 35% after ~13 min prior to decreasing sharply by 20 min. The sum of the water-soluble carbon yields in the initial 60 min was 93.2%. Furthermore, the Chromogen I yield also peaked at ~13 min with a value of 0.9%, and a subsequent decrease was observed by 20 min. The sum of the Chromogen I yields between 0 and 60 min was 1.6%.

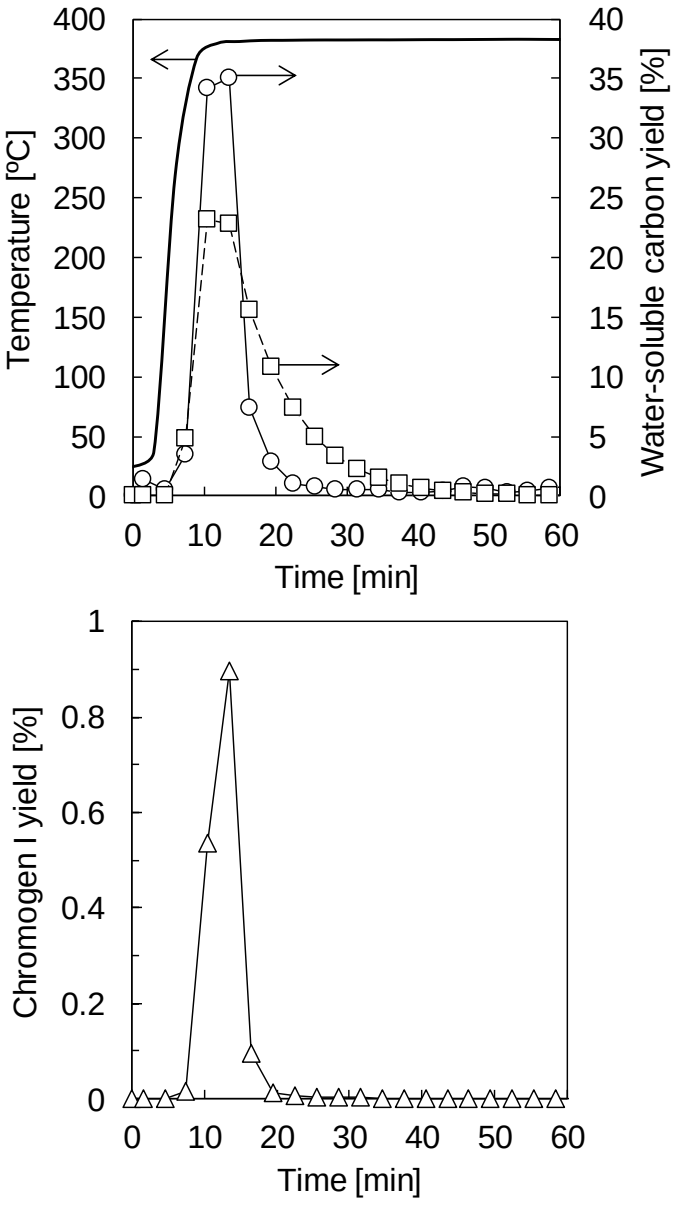

Fig. 5 Results of the chitin conversion reaction in high-temperature water (Run 3). The water-soluble carbon yield: (○) experimental and (□) calculated results.

As shown in Figure 6 (Run 4), the temperature reached 385 °C after ~7 min. The water-soluble carbon yield for this run showed a peak of 35% at ~10 min, and this was followed by a rapid decrease by 15 min. The sum of the water-soluble carbon yields in the initial 30 min was 90.2%. In addition, the Chromogen I yield also peaked at ~10 min, giving a value of 0.29%, after which point, a rapid decrease was observed within 15 min. The sum of the Chromogen I yields from 0 to 30 min was 1.4%.

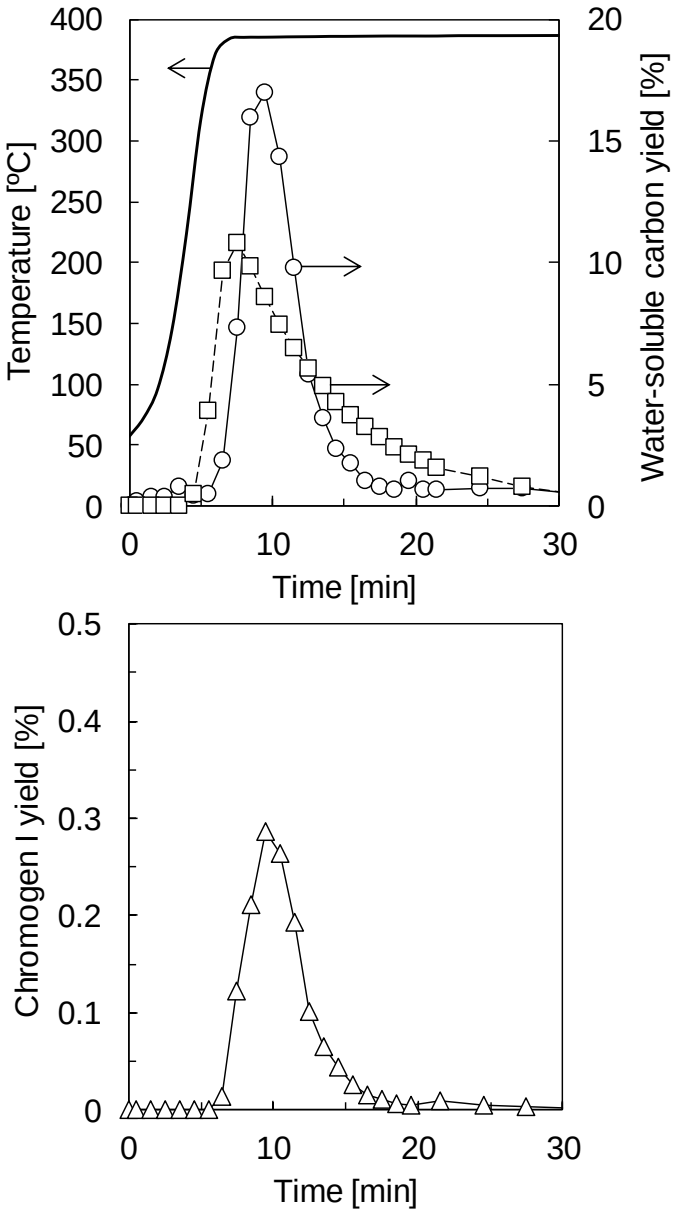

Fig. 6 Results of the chitin conversion reaction in high-temperature water (Run 4). The water-soluble carbon yield: (○) experimental and (□) calculated results.

Furthermore, as shown in Figure 7 (Run 5), the temperature reached 390 °C after ~9 min. In this case, the water-soluble carbon yield showed a sharp peak of 46% at ~8 min and then decreased rapidly by 17 min. The sum of the water-soluble carbon yields in the

initial 30 min was 86.7%. Moreover, the Chromogen I yield at ~8 min was 0.22%, although a rapid decrease was observed within 17 min. The sum of the Chromogen I yields between 0 and 30 min was 0.6%.

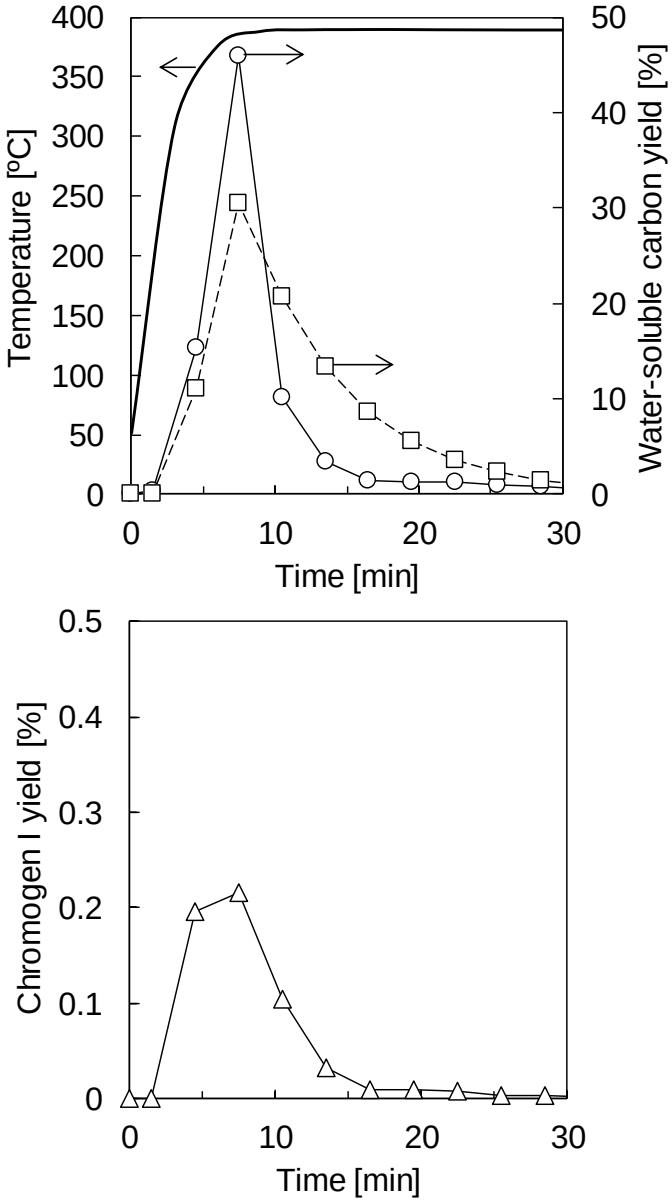

Fig. 7 Results of the chitin conversion reaction in high-temperature water (Run 5). The water-soluble carbon yield: (○) experimental and (□) calculated results.

To estimate the water-soluble carbon yield, a kinetic model was developed. The decomposition rate of chitin in high-temperature water was evaluated by assuming first order reaction kinetics for the water-soluble carbon yield, $W(t)$, at time t:

$$dW(t)/dt = k\,W(t) \quad (4)$$

where k is the overall reaction rate constant:

$$k = A \exp(E_a/RT(t)) \quad (5)$$

and where A is the preexponential factor, E_a is the apparent activation energy, R is 8.314 J K^{-1} mol^{-1}, and $T(t)$ is the temperature at time t in Figures 3–7. In this work, the kinetic parameters in eq. (5), i.e., A and E_a, were evaluated by fitting of the experimental data by nonlinear regression using Microsoft® Excel Solver. As a result, A and E_a were determined to be $10^{2.55}$ and 65.4 kJ mol^{-1}, respectively. The evaluated water-soluble carbon yields are shown in Figures 3–7, and it was apparent that the variation in yield with time was well represented by eqs. (4) and (5).

In our previous research using a batch type reactor, A and E_a were found to be $10^{6.76}$ and 108 kJ mol^{-1}, respectively [26]. In that previous example, we input a constant temperature into eq. (5), which was the final temperature after the temperature increase. However, in the current study, we also consider the temperature increase by introducing $T(t)$ into eq. (5). Furthermore, for the batch reactor, there is a possibility that the water-soluble products could be converted into water-insoluble products, and so the water-soluble carbon

yield would be inaccurate. In contrast, for the semi-batch reactor employed herein, the water-soluble products were immediately removed from the high-temperature conditions, thereby ensuring that any subsequent conversion to water-insoluble products would be negligible. The kinetic parameters obtained in this work should therefore be more accurate than those reported in our previous study. However, the reaction rate for the conversion of chitin powder to the water-soluble fraction is affected by the size of chitin powder particles and the loading conditions of the semi-batch reactor, and so elucidation of the effects of these factors is necessary for further optimization.

As shown in Figures 3–7, the water-soluble carbon yield and Chromogen I yields peaked earlier upon increasing the set temperature. It should be noted that the rate of the temperature rise increased with an increase in the final set temperature because a higher temperature of water was introduced into the reactor. As a result, the rate of chitin degradation was also enhanced at higher temperatures. In addition, the decreases in the water-soluble carbon yield and the Chromogen I yields after reaching their peak values became more pronounced upon increasing the final temperature due to the conversion of chitin reaching completion more rapidly.

Although ~90% of the chitin powder could be converted into water-soluble products, the low Chromogen I yields (i.e., <3%) indicate that various unknown products (~87%) were also present, and so it appears difficult to selectivity obtain a specific product from chitin by

high-temperature water treatment. We found that the highest yield of Chromogen I was obtained in Run 2 (360 °C); however, the differences in yields between 290 and 390 °C were small. These results render it difficult to determine the optimal conditions for production of the GlcNAc derivatives.

We propose two main reasons for the low yields obtained for the GlcNAc derivatives, namely an alteration in the chemical structure of chitin, and differences between the optimal temperatures for the dehydration of GlcNAc and the degradation of chitin.

In this first case (i.e., an alteration in the chemical structure of chitin), a broader peak was observed for the water-soluble carbon yield in Run 1 (290 °C) compared to those of higher temperatures Run 2–5, indicating that a quantity of chitin remained in the reactor for ~3 h. We previously reported that the degradation of chitin in high-temperature water in a conventional batch reactor proceeds not only via hydrolysis, but also via dehydration [26, 27]. This phenomenon could also occur in this case, and so the chemical structure of the chitin would be altered during the semi-batch treatment process. As a result, a low Chromogen I yield was obtained for Run 1 (290 °C).

In terms of the differences between the optimal temperatures for the dehydration of GlcNAc and the degradation of chitin, at temperatures >360 °C, the water-soluble carbon yields in Run 2–5 peaked within 20 min, indicating that the chitin powder was not exposed to high-temperature water for a long period of time. However, the yield of Chromogen I was

low (i.e., <3%). In a previous study into the dehydration of GlcNAc in high-temperature water, it was found that the reaction proceeded within 1 min at ~200 °C [19], with prolonged treatment leading to further decomposition. However, the degradation of chitin powder into water-soluble products required >1 h at ~300 °C, and so it could be estimated from the calculated reaction rate that chitin treatment at 200 °C would require approximately two days to achieve full conversion (eqs. (4) and (5)). These results indicate a temperature difference of 100 °C for the optimal temperatures between the dehydration of GlcNAc and the degradation of chitin. When a typical batch reactor is employed, it is difficult to achieve a rapid change in the reaction temperature. We therefore employed a semi-batch reactor for the degradation of chitin at temperatures >300 °C followed by dehydration at 200 °C by rapid cooling. Although we expected to obtain dehydration products such as Chromogen I in higher yields, a Chromogen I yield of <3% was obtained, likely due to the slow cooling rate of the semi-batch reactor. The cooling time required between 300 and 25 °C was estimated to be ~10 s by the residence time of the heat exchanger and the temperature of the T4 thermocouple. Although cooling was achieved in ~10 s, the reaction time required for the dehydration of GlcNAc to Chromogen I at 300 °C was estimated to be only 0.2 s based on a previous study [19]. As such, the decomposition of Chromogen I likely took place, thereby lowering the yields of the dehydration products, namely the GlcNAc derivatives. Further reductions in the cooling time would therefore be desirable to improve the yield of

Chromogen I.

4. Conclusions

We herein described the successful conversion of chitin into *N*-acetyl-D-glucosamine (GlcNAc) derivatives in high-temperature water at 290–390 °C and 25 MPa. Although the original chitin powder could be converted into a water-soluble fraction in ~90% yield, the highest yield obtained for Chromogen I was 2.6%. In addition, although this yield is lower than those reported for previous systems based on the use of organic solvents and/or ionic liquid, this novel method employs only water. We therefore demonstrated successfully that the non-catalytic conversion of chitin in high-temperature water is an environmentally benign route to GlcNAc derivatives. Furthermore, the preexponential factor A and the apparent activation energy E_a of the chitin powder conversion into the water-soluble fraction were determined to be $10^{2.55}$ and 65.4 kJ mol^{-1}, respectively. Using the obtained kinetic parameters, A and E_a, the experimental water-soluble carbon yield was well explained. Further work is required to improve the semi-batch reactor and shorten the cooling time, which would be expected to increase the yields of the GlcNAc derivatives.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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