SUPERCRITICAL WATER TREATMENT AND ENZYMATIC SACCHARIFICATION OF CHITIN BIOMASS

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We examined the effect of supercritical water treatment on enzymatic saccharification from crustacean chitin to *N*-acetylglucosamine (GlcNAc) and *N,N'*-diacetylchitobiose ($(GlcNAc)_2$, chitin structural dimeric unit). By the combination of supercritical water treatment and enzymatic saccharification, we succeed to convert chitin into $(GlcNAc)_2$. In addition, by grinding chitin using a mill after the supercritical water treatment, we achieved over 90% of the degradation ratio by enzymatic saccharification.

INTRODUCTION

N-acetylglucosamine (GlcNAc) produced from crustacean chitin (α-chitin) possesses a therapeutic effect on arthritis. *N,N'*-diacetylchitobiose ($(GlcNAc)_2$, chitin structural dimeric unit) is expected to be a source for oligosaccharides production. Although the market is rapidly expanding, the production of GlcNAc and $(GlcNAc)_2$ from crab shells takes a large number of processes and strong acid because of their hard crystallinity and insolubility of α-chitin. There are some reports in order to obtain $(GlcNAc)_2$ and GlcNAc from chitin by just the sub- and supercritical water treatment because the hydrolysis reaction proceeds without any catalyst in sub- and supercritical water [1,2]. However, GlcNAc and $(GlcNAc)_2$ were not obtained because the decomposition of these compounds proceeded at the same time of the hydrolysis of chitin. In this study, we investigated suitable conditions of supercritical water treatment that the decomposition of GlcNAc structure does not occur and promotes the enzymatic saccharification.

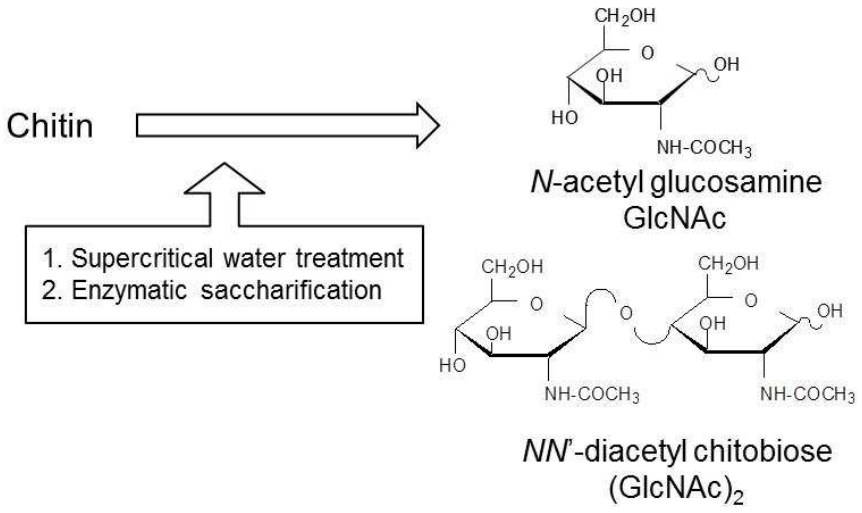

Figure 1. Scheme of this work

MATERIALS AND METHODS

Crab chitin (Yaizu suisankagaku industry) was used for material. For the enzymatic saccharification, crude enzyme from *Streptomyces griseus* was used as chitinase. The enzyme produces $(GlcNAc)_2$ as a major product from chitin.

Treatment of chitin in supercritical water was conducted in a SUS 316 tube reactor of 6 cm^3 in volume. After chitin flakes 0.2 g and water 3 g were loaded in the reactor, the reactor was submerged into a molten-salt bath (KNO_3–$NaNO_3$) kept at 400°C. In this work, the reaction starting point was set at the moment when the reactor was submerged into the molten-salt bath; therefore, the reaction time includes the initial heating period. After a given reaction time, the reactor was taken out from the molten-salt bath and rapidly quenched in a water bath to cool down to room temperature. After cooling, the products were collected from the reactor and divided into the water-soluble and solid fractions (chitin) with a membrane filter. After supercritical water pretreatment, the chitin dried at 90°C for 24 h.

The enzymatic saccharification of chitin was conducted as follows. 20 mg of chitin was mixed with 1.8 mL of 10 mmol/L phosphate buffer (pH 6.0) and 0.2 mL of 10 mg/mL enzyme diluted with 10 mmol/L phosphate buffer. The reaction mixture was shaken at 1400 rpm at 40 °C, and 0.4 mL of the mixture was harvested at the appropriate time. The harvested reaction solution was filtered after boiling for 10 min, and centrifuged [3].

The HPLC system consisted of an ELSD detector. GlcNAc and $(GlcNAc)_2$ were separated on a Shodex SUGAR KS-802 column using ultrapure water as the mobile phase. Yield was calculated from the area of the peak of the enzymatic reaction mixture and two standards of $(GlcNAc)_2$ and GlcNAc. The degradation ratio was divided by the chitin concentration.

RESULTS & DISCUSSION

Figure 2 shows degradation ratio from chitin to GlcNAc and $(GlcNAc)_2$ by enzymatic saccharification of (a) untreated and (b) supercritical water treated chitin at 400 °C for 1 min. Some experiments were repeated two to three times to quantify run-to-run variability. For untreated chitin, degradation ratio at 72 h was only 5%. The degradation ratio of supercritical water treated chitin reached 37%. From Figure 2, the enzymatic saccharification would be completed at a reaction time of 48 h. From XRD analysis of the chitin, the maximum peak shifted to lower degree by the supercritical water treatment, indicating that the lattice plane spacing became wide. Therefore, the enzyme would move between the lattice plane spacing easily and the enzymatic hydrolysis would proceed efficiently after the supercritical water treatment. We also analyzed the aqueous solution recovered after supercritical water treatment; however, GlcNAc and $(GlcNAc)_2$ were not obtained.

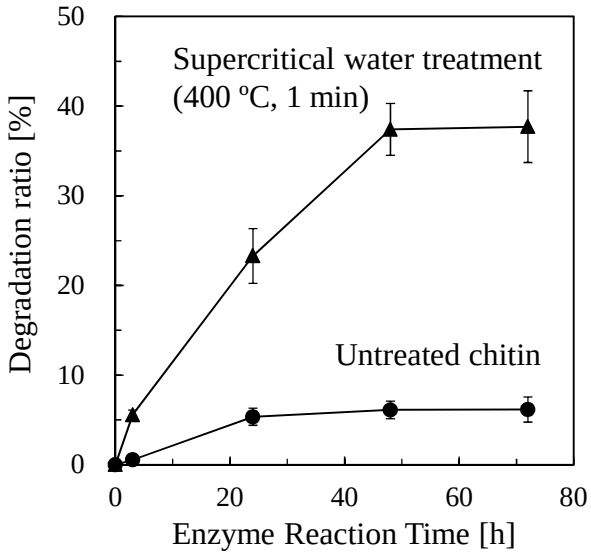

Figure 2. Effect of supercritical water treatment on enzymatic saccharification of chitin

Figure 3 shows the effect of supercritical water and grinding treatment by a ball mill on enzymatic saccharification of chitin. As shown in Figure 2, the degradation ratios of untreated and supercritical water treated chitin were 5 and 37%, respectively. In absence of water condition at 400 ˚C and 1 min, namely thermal treatment, the chitin flacks changed to a black solid like char. The degradation ratio of the black solid was 0%, indicating that it was not degraded by enzyme. Therefore, we could confirm the necessity of supercritical water for treatment of chitin. The degradation ratio after grinding (30 min) was 60% [3]. In addition, for case of grinding the chitin by a mill after the supercritical water treatment, we achieved over 90% of the degradation ratio.

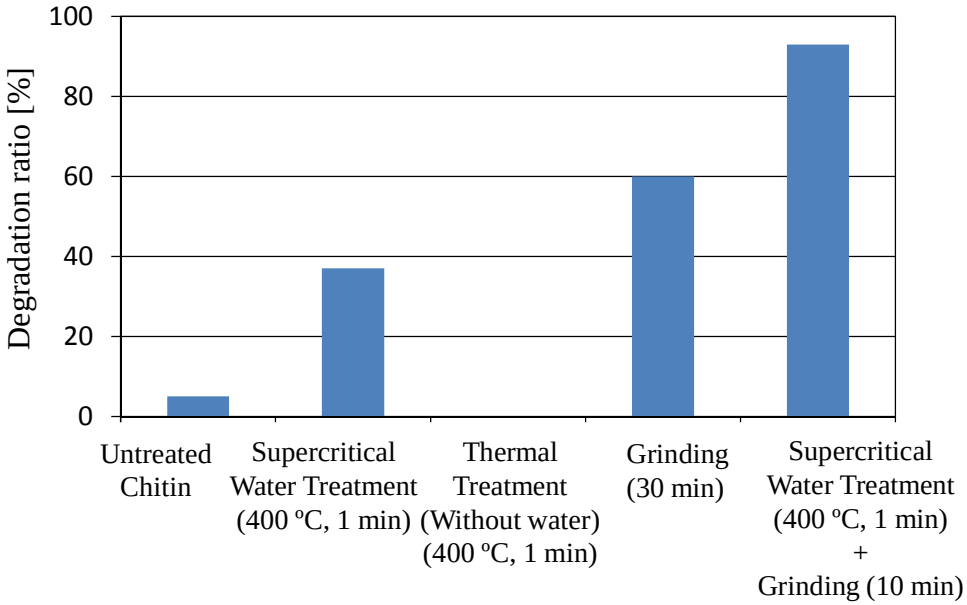

Figure 3. Effect of pretreatments on enzymatic saccharification of chitin

CONCLUSION

We found that the enzymatic saccharification of chitin into GlcNAc and $(GlcNAc)_2$ was proceeded by the supercritical water pretreatment. In addition, by grinding chitin using a mill after the supercritical water treatment, we achieved over 90% of the degradation ratio.

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