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Effect of Ultrasounic Irradiation on Enzymatic Saccharification of Cellulose

セルロースの酵素糖化に及ぼす超音波照射の影響

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1. Introduction

The cellulose in wood is the most abundant biomass on earth. Enzymatic hydrolysis of cellulose produces soluble sugars. This process is very attractive because it is simple and milder operating conditions, and hardly makes detrimental harmful by-products for next process such as fermentative microorganism. However the enzymatic saccharification of woods is of low efficiency and slow reaction rate because the cellulose in wood has high crystallinity and low specific surface area. Ultrasonic irradiation in water can break aggregation solids and enhance mass transfer of suspended solids.

In this study, the effects of equipment and operation conditions of ultrasonic irradiation on enzymatic saccharification of cellulose for needle unbleached kraft pulp and microcrystalline cellulose powders were examined.

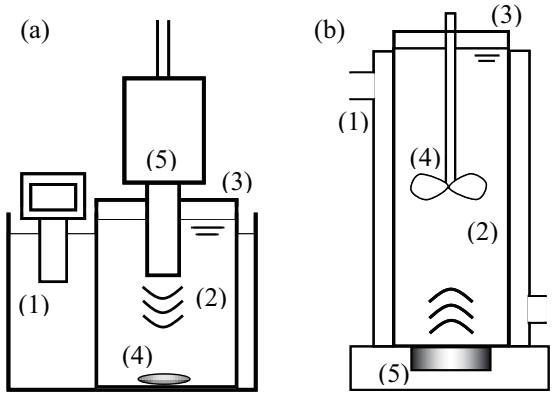
2. Experimental

Fig. 1 shows outline of experimental apparatus. Two types of ultrasonic devices were used. One was the horn-type transducer at 20 kHz, which was installed vertically into the reactor. The sample volume was 800 mL and mixed by using magnetic stirrer from bottom. The other was the plate-type transducer at 28 kHz or 500 kHz, which was attached at reactor bottom. The sample volume was 350 mL and mixed by using stirring rod from top. In both apparatus, the ultrasonic intensity was varied. The ultrasonic intensity was measured by calorimetric method.

The substrates were needle unbleached kraft pulp (NUKP) and microcrystalline cellulose powders (CC31). The initial concentrations of substrate for NUKP and CC31 was 1.5 g/dm^3, respectively. Meicelase (provided by Meiji Seika Ltd.) was used as enzyme. The concentrations of enzyme were 0.4 g/dm^3. The reaction was carried out acetate buffer solution of pH = 4.8 and its concentrations for NUKP and wood powder were 0.1 and 0.01 M, respectively. The temperature was kept at 45 °C. The concentration of total sugar was estimated by the phenol-sulfuric acid method.

3. Results and discussion

Fig. 2 shows the change in total sugar concentration with time for different frequencies. The sample is NUKP and enzyme is Meicelase. The effective input power to transducer was 30 W.



(1) Const. Temp. Bath (4) Stirrer
(2) Sample (5) Ultrasonic Transducer
(3) Vessel

Fig. 1 Outline of Experimental apparatus

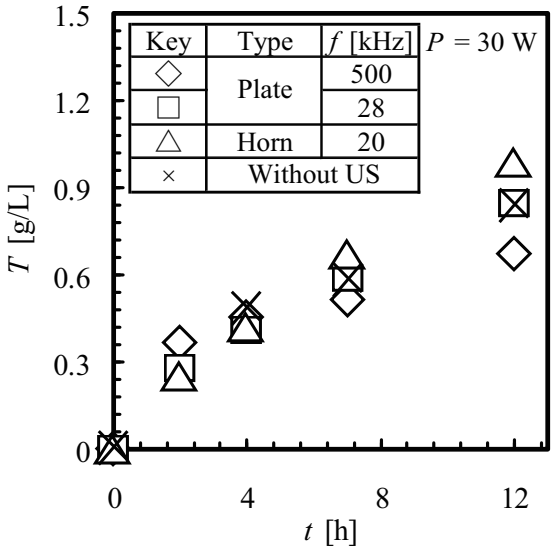


Fig. 2 Change in total sugar concentration with time

The concentration of total sugar increases with time. In the cases of horn-type transducer at 20 kHz and plate-type transducer at 28 kHz, the enzymatic saccharification was accelerated. On the other hand, the case of plate-type transducer at 500 kHz, the enzymatic saccharification was inhibited. It is considered that the enzyme is inactivated by OH radical generated from pyrolysis water due to ultrasonic cavitation. The I_3^- concentrations generated from KI solution for 500 kHz were much higher than those for 20 and 28 kHz.

Fig. 3 shows the effect of ultrasonic intensity on the ratio of total sugar concentration for low frequency ultrasound. The ordinate indicates the ratio of total sugar concentration at 12 hours with and without ultrasound. The ultrasonic intensity was measured by using caloric methods. The ratio of total sugar concentration increases linearly with ultrasonic intensity. Since the low frequency ultrasound has large-amplitude compared with high frequency, it is considered that the ultrasonic irradiation physically breaks substrate surface and accelerates diffusion of cellulase into substrate.

The horn with ultrasonic transducer at 20 kHz and the plate of ultrasonic transducer at 42 kHz were used. **Fig. 4** shows the effect of superposition of ultrasound on ratio of total sugar concentration. The superposition of ultrasound field was appeared synergy effect for the enhancement of enzymatic saccharification.

Fig. 5 shows the effect of crystallinity of substrate on ratio of total sugar concentration. The enhancement of ultrasound for CC31 was higher than that for NUKP. This results indicate that ultrasonic irradiation is effective for cellulose with high crystallinity.

4. Conclusion

The effects of equipment and enzyme conditions of ultrasonic irradiation on enzymatic saccharification of cellulose were examined. The following results were found.

1) The saccharification of cellulose is enhanced by using ultrasonic irradiation.
2) The enhancement of cellulose saccharification at low frequency is higher than that at high frequency.
3) The superposition of ultrasound field was appeared synergy effect for the enhancement of enzymatic saccharification.
4) The enhancement of cellulose saccharification for high crystallinity is higher than that for low crystallinity.

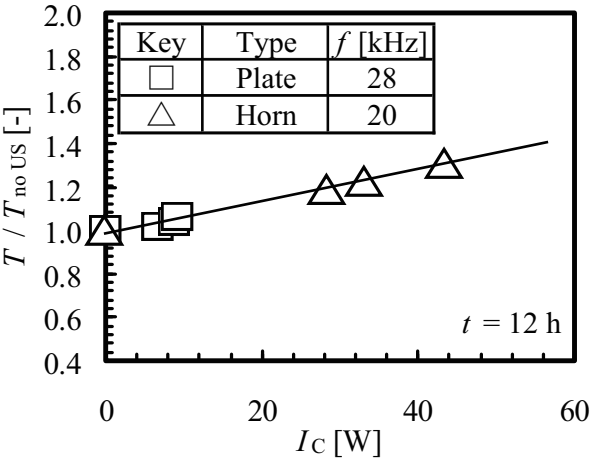


Fig. 3 Effect of ultrasonic intensity on ratio of total sugar concentration for low frequency ultrasound

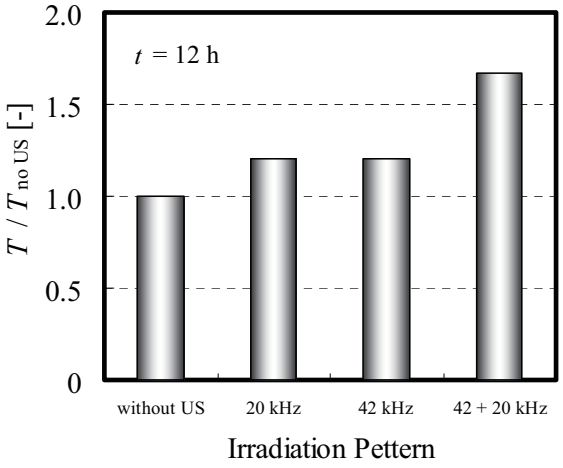


Fig. 4 Effect of superposition of ultrasound on ratio of total sugar concentration

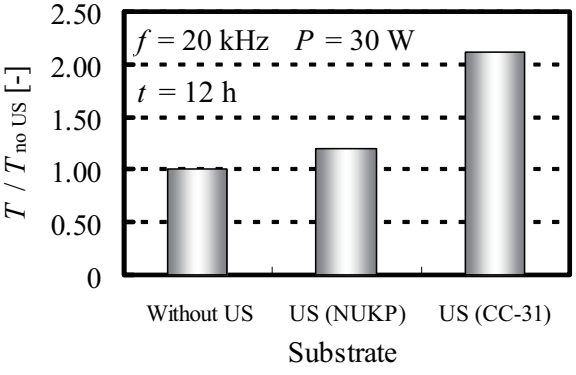


Fig. 5 Effect of crystallinity of substrate on ratio of total sugar concentration