論 文 の 内 容 の 要 旨

Title of Dissertation: Effects of Lignocellulose Pretreatment Using Surfactants on Enzymatic Saccharification
（界面活性剤を用いたリグノセルロースの前処理が酵素糖化に及ぼす影響）

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1. Introduction

Currently, attention to the utilization of lignocellulose which account for roughly 20% of the terrestrial feedstock carbon storage as a sustainable feedstock for biorefinery has been growing. Saccharification of cellulose in the lignocellulose by saccharification enzymes, namely cellulase (i.e. CBHs; cellobiohydrolases, EGs; endo-glucanases and β-glucosidase) is a key step of biorefinery of lignocellulose. However, the intrinsic three-dimensional cell-wall structure of lignocellulose composed of cellulose microfibril aggregates linked with a lignin and hemicellulose matrix strongly interferes with the access of cellulase to cellulose (Fig.1) and lowers the efficiency of synergetic interaction of cellualse (Fig. 2).

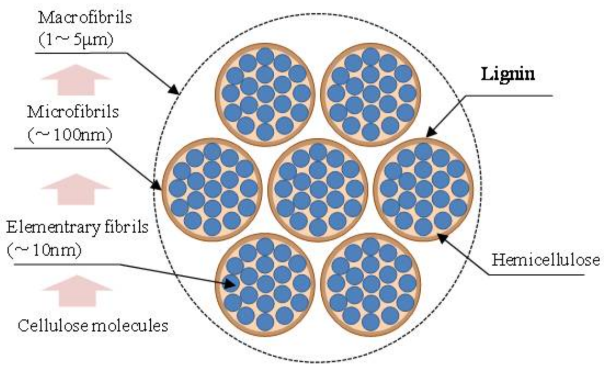

Fig.1 Three-dimensional structure of lignocellulose

Therefore the increase of cellulase accessibility to cellulose has been the main technical issue and various pretreatments combined with severe conditions (i.e. high pressure, temperature and dosage of chemicals) are introduced to achieve sufficient saccharification of the cellulose in lignocellulose. However, because economics of biorefinery are strongly affected by severity of pretreatment and dosage of enzyme loading, economic commercial scale process is yet to be proposed. Among a number of studies dealing with these technical and economical issues, the addition of surfactants to saccharification process has been attracting a great deal of attention as a possible solution for above issues.

Most of previous studies in this field have focused on the effect of surfactants on the interaction between cellulase and lignin and reported that surfactants lower the non-biospecific and irreversible adsorption of cellualse onto lignin[1), 2)]. Nevertheless, a number of studies qualitatively assessed the various roles of surfactants on the saccharification, the major role of surfactants controlling the behaviors of cellulase and the saccharification efficiency was not fully understood. Besides, structural effects of surfactants on lignocellulose are rarely discussed so far. Knowing these things is very important to maximize the surfactant's effects on saccharification of cellulose in lignocellulose.

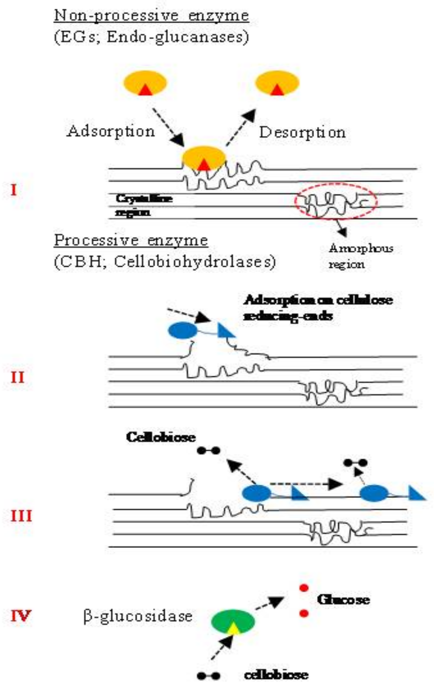

Fig. 2 Steps for synergetic interaction cellulase (Saccharification)

To propose a better way for effective utilization of lignocellulose by surfactants, the author clarified **i)** the major role of a surfactant on the saccharification, **ii)** the structural changes of lignocellulose by a surfactant and their effects on cellulase adsorption (equilibrium and rate) and saccharification and proposed **iii)** the quantitative model which describes effects of a surfactant on saccharification of cellulose in lignocellulose in this work.

2. The major role of Tween 20 on cellulase reaction

2.1 Material and methods

The author prepared lignocellulose of different lignin contents (R: 31, P1: 25, P2: 15, P3:10, P4: 5%) and microcrystalline cellulose (Avicel) as samples. Non-ionic surfactant, Tween 20 was used as a model surfactant. Knowing the adsorption behaviors of Tween 20 on samples is necessary to interpret the reason for the enhancement of cellulose conversion by surfactants. Therefore, batch Tween 20 adsorption was carried out at room temperature for 24hrs. As shown in Fig.2, the author assessed the role of Tween 20 on liquid phase (cellobiose hydrolysis; step IV) and solid surface interaction (cellulose hydrolysis; step I-III), respectively. For liquid phase interaction, batch cellobiose hydrolysis (50˚C) with and without Tween 20 was performed. For solid surface interaction, analysis of Tween 20's effects on cellulase reaction is not easy since adsorption and hydrolysis occur simultaneously. Therefore the author examined the effects of Tween 20 on cellulase adsorption and cellulose conversion, respectively. Batch cellulase adsorption experiment (0˚Cfor 6hrs) and enzymatic hydrolysis (50˚C for 72hrs) were carried out.

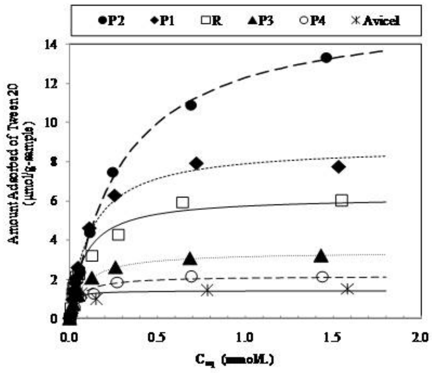

Fig. 3 Tween 20 adsorption isotherms

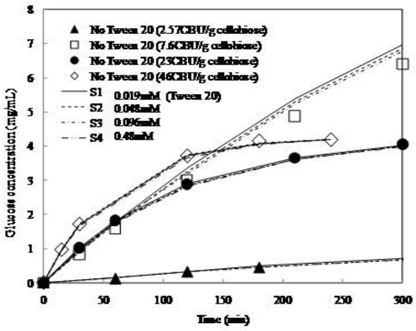

Fig.4 Effect of Tween 20 on cellobic hydrolysis

2.2 Results and Discussion.

As shown in Fig.3, both lignocellulose and pure cellulose showed Langmuir-type adsorption isotherms of Tween 20, which means that Tween 20 is adsorbed to form monomers and that no hemi-micelles or admicelles are formed on the adsorbent surface. Considering that Tween series adsorb on the adsorbent by H-bonding with oxygen ethylene of the hydrophilic head and hydrophobic interaction with hydrophobic tails, it can be said that adsorbed Tween 20 as a monomer on surfaces by H-bonding and hydrophobic interaction and free Tween 20 (monomer and/or micelle) in solution affect the interactions between enzymes and lignocellulose.

Fig. 4 shows the effects of Tween 20 on cellobiose hydrolysis by β-glucosidase (step IV). Under the various enzyme/ substrate ratio, Tween 20 had no effects on liquid phase interaction. Fig. 5 shows the Tween 20's effects on cellulase adsorption on lignocellulose at around 0˚ C at which the catalytic activity of cellulase is negligible (without hydrolysis). Tween 20 increased amount adsorbed of cellulase onto both samples P1 (high lignin contents) and P4 (low lignin contents).

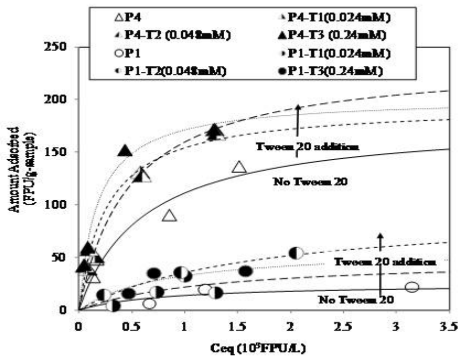

Fig.5 Effect of Tween 20 on cellulase adsorption

Cellulase adsorption (eq.1) and cellulose conversion (eq.2) during hydrolysis were examined using previously reported empirical equations[3] as below. During the hydrolysis of cellulose, Tween 20 also increased the

$$\frac{d(E_a/E_0)}{dt} = k_1\left(\left(\frac{\overline{E_{a0}}}{E_0}\right)\left(\frac{C}{C_0}\right)^{\alpha} - \left(\frac{E_a}{E_0}\right)\right) \quad (1)$$

$$-\frac{dC}{dt} = \frac{k_2 C\left(\frac{C}{C_0}\right)^{\beta}\left(\frac{E_a}{E_0}\right)}{1+\left(\frac{C_0 - C}{K_I}\right)} \quad (2)$$

E_a: Amount of adsorbed cellulase (FPU)
E_0: Amount of injected cellulase (FPU)
$\overline{E_a}$: The equilibrium amount of cellulase (FPU)
C: Concentration of cellulose (g/L)
C_0: Initial concentraion of cellulose (g/L)
k_1: rate constant (hr^{-1})
k_2: rate constant (hr^{-1})
K_I: Inhibition constant (L/g)
α, β: constant (-)

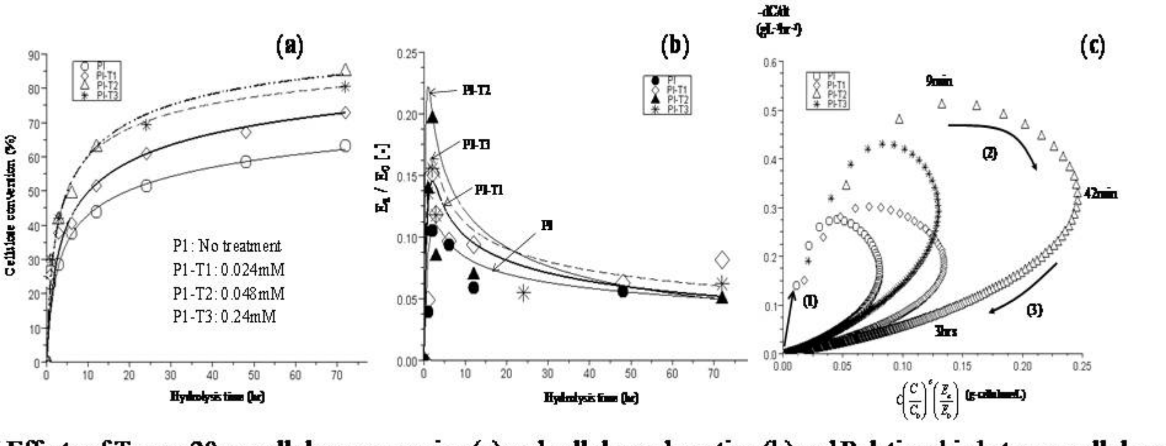

Fig. 6 Effects of Tween 20 on cellulose conversion (a) and cellulase adsorption (b) and Relationship between cellulose conversion rate and parameters related with availability of cellulose to cellulase (c)

cellulose conversion as well as the amount adsorbed of cellulase onto cellulose as shown in Fig. 6a, b.

Analysis using empirical equations showed that Tween 20 increased cellulose conversion rate not by increasing the cellulase activity itself but by increasing the adsorption site (cellulose surface) of cellulase (Fig 6c).

3. Structural effects of Tween 20 on lignocellulose

3.1 Material and methods

To verify the above inference that is Tween 20 increases the accessible cellulose surface, the author treated various samples of different properties (i.e. lignin contents and crystallinity) with different concentration of Tween 20 in a stirred vessel for 24-48hrs and also monitored the macroscopic structural change of specimens. Moreover, x-ray diffraction (XRD) and differential scanning calorimetry (DSC) analysis were carried out for inner structure study.

3.2 Results and discussion

It was found that Tween 20 treatment contributed to the cell wall collapse of most of samples as shown in Fig. 7 except for those with high lignin contents and high crystallinity. Through the XRD analysis, no substantial changes in crystallite size of samples due to cell wall collapse were observed. This means that cellulose elementary fibrils in lignocellulose were rarely affected by Tween 20. DSC analysis showed the changes in pore water contents by Tween 20 treatment. As shown in Fig. 8, Cell wall collapse by Tween 20 contributed to the formation of 10- to 50-nm pores. Moreover, non-freezing bound water, generally considered as pore water which does not freeze due to the intimate hydrogen-bond to a cellulose chain, also increased largely. From the DSC analysis, it can conclude that Tween 20 treatment contributed to water intrusion into cellulose microfibrils in lignocellulose and increase of accessible cellulose surface.

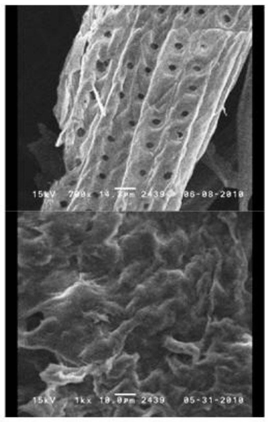

Fig. 7 Cell wall collapse of lignocellulose by Tween 20 (upper: not treated, down: Tween 20 treated)

Fig. 9 shows the structural effects of Tween 20 on cellulase adsorption amount and rate.

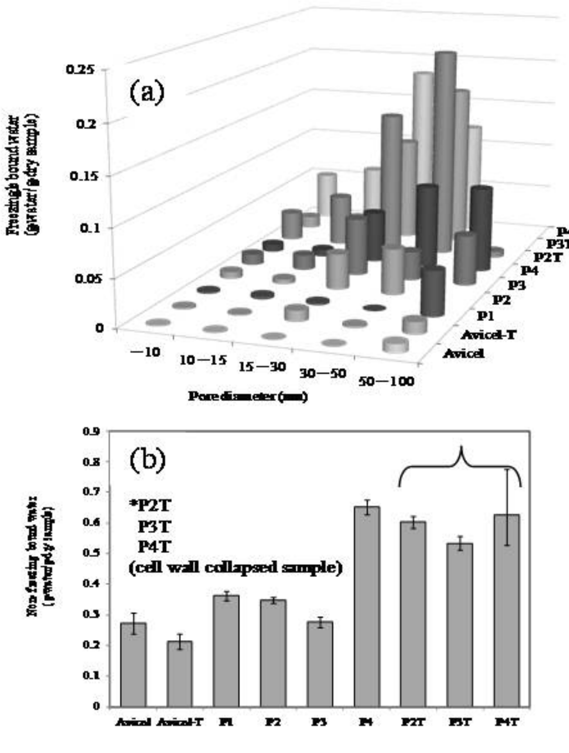

Fig. 8 Variation of pore water by Tween 20 treatment (a)Freeze bound water (b)Nonfreeze bound water

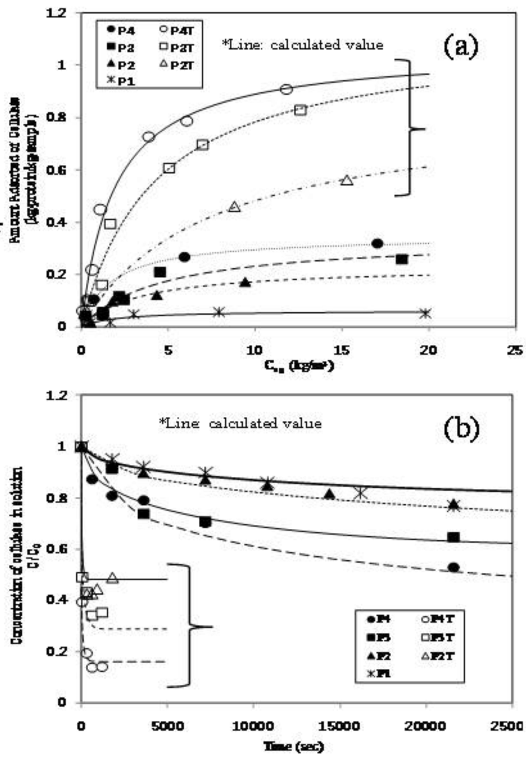

Fig. 9 Variation of cellulase adsorption (a) Amount (b) Adsorption rate by Tween 20 treatment

Cellulse adsorption rate was quantified using the general numerical diffusion model[4)] for cylindrical particle as below. Cell wall collapse with Tween 20 treatment not only increased the monolayer saturation amount of adsorbed cellulase about 3–3.6 times (Fig. 9a) but also increased the cellulase adsorption rate (D_e/r^2) about 160–880 times (Fig. 9b). Considering the dimensions of cellulase (EGI: 10–20 nm, CBHII: 5–10 nm), it is thought that 10- to 50-nm size pores newly formed by Tween 20 treatment are sufficient to enhance accessibility of cellulase into cellulose microfibrils in lignocellulose.

$$\gamma \frac{\partial q}{\partial t} = D_e \left(\frac{\partial^2 C}{\partial r^2} + \frac{1}{r} \frac{\partial C}{\partial r} \right) \quad (3)$$

q: Amount of cellulase adsorbed onto the unit sample (kg-protein/kg-sample)
D_e: Effective diffusion coefficient (m^2/s)
C: Cellulase concentration in solution (kg/m^3)
r: Radius of diffusion (m)
γ : loading rate of sample (kg/m^3)

4. Construction of cellulose conversion model with consideration of Tween 20's effect

Through this study the author verified that Tween 20 increased the accessible cellulose surface and it contributed to enhancement of cellulase adsorption onto cellulose. Judging from results obtained, the structural effects of Tween 20 can be shown in Fig. 10. Water intrusion into cellulose microfibrils by Tween 20 exposes cellulose elementary fibrils to cellulase reaction. Hence, the cellulose radius for cellulase reaction is decreased. Cellulose conversion model based on the physical evidences obtained from this work was constructed as below equation.

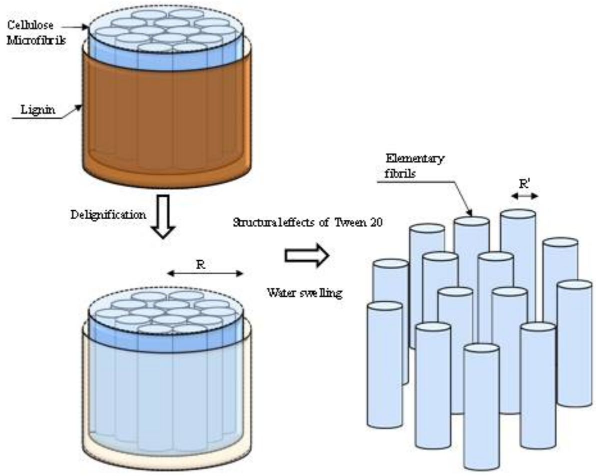

Fig. 10 Structural effect of Tween 20

$$\frac{d\alpha}{dt} = k_a \cdot \exp\left(-k_b \cdot \frac{\alpha}{(1-\alpha)} \right) \quad (4)$$

α: conversion ratio (-)
k_a: parameter related with adsorbed cellulase and radius of cellulose fibril
k_b: parameter related with retardation of cellulase reaction

5. Summary

Although application of surfactants has been attracting a great attention, conventional pretreatment methods are still limited to injection as a supplement of saccharification for pretreated lignocellulose. However, the author showed for the first time that structural effects of Tween 20 contributed to significant enhancement of cellulase adsorption and saccharification. The author believes that a new aspect of Tween 20's roles on saccharification of cellulose in lignocellulose proposed in this work can contribute to expansion of surfactant application field for biorefinery and effective saccharification of cellulose in lignocellulose.

6. Reference

1) Eriksson, T., Börjesson, J., Tjerneld, F., Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. Enzyme Microbial. Tech. 31, 353-364 (2002)
2) Kumar, R., Wyman, C.E., Effect of additives on the digestibility of corn stover solid following pretreatment by leading technologies. Biotech. Bioeng.102, 1544-1557 (2009)
3) Liao, W., Liu, Y., Wen, Z.Y., Kinetic modeling of enzymatic hydrolysis of cellulose in differently pretreated fibers from dairy manure, Biotech. Bioeng.101, 441-451 (2008)
4) Xiu, G., Nitta, T., Li, P., Jin, G., Breakthrough curves for fixed-bed adsorbers: Quasi-lognormal distribution approximation. AIChE Journal 43, 979-985 (1997)