1. INTRODUCTION

Due to global warming caused by excessive use of fossil resources, renewable biomass resources will become more important in the future as alternatives to fossil resources. In addition, supercritical fluid technology has been considered to be an environmentally-benign treatment. Therefore, its technology was applied for a conversion of biomass to useful fuels and chemicals so as to mitigate the environmental loading.

For example, supercritical water treatment of lignocellulosics has demonstrated that they can be hydrolyzed to be lignin-derived products for useful aromatic chemicals and carbohydrate-derived products such as polysaccharides, oligosaccharides and monosaccharides of glucose, mannose, xylose for subsequent ethanol fermentation. If this treatment is prolonged, lignocellulosics were found to be converted to organic acids such as formic, acetic, glycolic and lactic acids which can be converted to methane for biofuel. If alcohols such as methanol, ethanol etc. are used instead of water, some other useful products were achieved and its liquefied products were found to have a potential for liquid biofuel.

In this study, therefore, our research achievements in supercritical fluid technology of biomass will be introduced for clean and green chemistry for a sustainable environment.

Keywords: Supercritical Fluid Technology, Liquid Biofuel, Biomethanol, Biomethane, Biodiesel, Lignocellulosics
2. LIQUID BIOFUEL FROM WOODY BIOMASS IN SUPERCRITICAL ALCOHOL TECHNOLOGY

Compared to petroleum, biomass resources from lignocellulosics are so bulky and difficult to handle, transport and reserve. Therefore, liquefaction of them offers ample opportunities for the advanced utilization. In this study, the liquefaction of woody biomass was studied by supercritical alcohol treatment to obtain liquid fuels and useful chemicals. Obtained liquid products can be used directly as liquid fuel because alcohol itself is a good fuel. Thus, 100% biomass-based liquid fuel and chemicals can be created when using a variety of bioalcohols.

On the work using methanol, ethanol, 1-propanol and 1-butanol, more than 90% of wood was decomposed and liquefied in any alcohol at 350°C as shown in Fig. 2 [2]. In case of methanol, however, a prolonged treatment time of about 30min was required for the conversion of wood. Therefore, the use of 1-octanol and 1-decanol was further studied for the possibility of wood liquefaction to achieve a higher conversion in more moderate reaction conditions [3]-[5].

Under the condition of 350°C, all three wood components, cellulose, hemicelluloses and lignin, were decomposed efficiently in 1-octanol and 1-decanol as well as in other alcohols. Thus, as shown in Fig. 3, more than 90% of wood was liquefied in them. Moreover, alcohols with longer alkyl chain exhibited a faster wood conversion. Among the alcohols used in this study, 1-octanol gave the fastest conversion of wood, achieving about 95% liquefaction of the whole wood conversion only by 3min treatment.

In such a shorter reaction time, high molecular weight products (about 10,000-30,000 in molecular weight) could be obtained as a liquid form. In addition, molecular weight distribution of the products could be regulated by changing the kind of alcohol and/or reaction conditions (Fig. 4).

These results imply that the supercritical alcohol treatment can convert woody biomass into biofuel and also sophisticated biodegradable materials thorough high energy efficiency.

For the development of applicable biofuel from woody biomass, its feasibility is necessary to be carried out for the obtained various alcohol-soluble portions, especially for octanol-soluble portion, by studying their ignitabilities and physical properties such as kinetic viscosity and pour point [6].

Fig. 2 A photograph of various alcohol-soluble portions achieved by supercritical alcohol treatments (From the left, methanol-, ethanol-, 1-propanol-,1-butanol- and 1-octanol-soluble portions in this order) [5]

Fig. 3 Changes in the residue of beech wood as treated in supercritical alcohols at 350°C [5]
3. BIOETHANOL FROM LIGNOCELLULOSICS BY SUPERCRITICAL WATER TECHNOLOGY

3.1 Supercritical water treatment of lignocellulosics to sugars

In this research, supercritical water treatment was applied to obtain bioethanol and useful chemicals from carbohydrates (cellulose and hemicellulose) and lignin in lignocellulosics, respectively, as shown in Fig. 5 [7]-[10]. To realize this process, the treatment condition must be optimized for obtaining fermentable saccharides such as polysaccharides, oligosaccharides and monosaccharides from lignocellulosics. Therefore, in this study, lignocellulosics decomposition by hot compressed water including supercritical water and subcritical water was performed. In addition, supercritical water and subsequent subcritical water treatments were also carried out to increase the yield of saccharides which can be used as substrates for bioethanol production [11].

Japanese beech (Fagus crenata) and Japanese cedar (Cryptomeria japonica) were used as a lignocellulosic biomass. The treatments were conducted with the flow-type supercritical fluid biomass conversion system. The treated sample was, then, separated to water-soluble portion, precipitates, methanol-soluble portion and hot compressed water-insoluble residue. Each fraction was analyzed by an appropriate analyzer; the precipitates and the hot compressed water-insoluble portion were analyzed by X-ray diffractometry. The water-soluble portion and precipitates were hydrolyzed by sulfuric acid (3.5%, 114°C, 1 h). The hydrolyzed samples were, then, analyzed by high performance liquid chromatography to determine the yield of glucose, fructose, arabinose, galactose, mannose and xylose.

As a result, in all treatment temperatures, the water-soluble portion was increased with treatment time, while the hot compressed water-insoluble portion was decreased. The precipitates were obtained only by the treatments in over 310°C. The dried precipitates showed cellulose II type of crystalline structure which is usually prepared by recrystallization of dissolved cellulose. This result, therefore, suggests that the precipitates would be dissolved in hot compressed water (over 310°C) but aggregated to be the precipitates in ordinary water.

After the hydrolysis of water-soluble portion and precipitates, around 20% in yield of monosaccharides from hemicellulose
(arabinose, galactose, mannose and xylose) could be obtained by all treatment temperatures. On the other hand, the monosaccharides from (glucose and fructose) ranged from 19% to 37% in yield by treatment over 330°C, while less than 10% of those was obtained by the treatment in lower than 320°C. Thus, treatments over 330°C could provide much more saccharides from Japanese beech than those below 330°C. These results suggest that crystalline cellulose and non-crystalline hemicellulose can be simultaneously hydrolyzed by treatment over 330°C due to cleavage of hydrogen bonds in water and cellulose.

However, it was also found that fragmentation reaction of saccharides is accelerated by increasing treatment temperature, with decreasing the yield of fermentable saccharides. Thus, we attempted to apply the combined process of the short supercritical water treatment and the following subcritical water treatment to inhibit fragmentation reaction and increase the yield of hydrolyzed products. As a result, this combined treatment was able to control the reaction effectively, and to increase the yield of hydrolyzed products with inhibiting fragmentation reaction [11].

To optimize the supercritical water treatment of lignocellulosics, we will have to conduct a study about the influence of treatment pressure on decomposition behavior of lignocellulosics. Characterization of oligomeric lignin-derived products in the methanol-soluble portion is also necessary to realize its effective utilization.

3.2 Inhibitors for ethanol fermentation in the lignocellulosics hydrolysate

Recently some studies on the supercritical water treatment for lignocellulosics have been carried out to obtain the fermentable water-soluble (WS) portion, as discussed in a previous section. However, the water-soluble portion obtained contains not only fermentable sugars but also some furan compounds such as furfural and 5-hydroxymethylfurfural, which are formed by the degradation of sugars, and various phenolic compounds, which are formed by degradation of lignin [8]-[9]. Although the quantities of these compounds in the hydrolysate are very little, these compounds inhibit the ethanol fermentation of the sugars by the microorganisms. The object in this study, therefore, is to improve the ethanol fermentability of the water-soluble portion obtained by supercritical water treatment of lignocellulosics.

Wood charcoals prepared at various temperatures were applied to remove the inhibitors for ethanol fermentation in the water-soluble portion obtained by supercritical water treatment of lignocellulosics. The treated water-soluble portions were fermented with the yeast, *Saccharomyces cerevisiae* to evaluate the effects of the wood charcoal treatment. *Candida shehatae* or *Pachysolen tannophilus* which can ferment not only hexose but also xylose were also used for ethanol fermentation of the treated water-soluble portions. Inhibitors, sugars and ethanol in the water-soluble portion were analyzed by HPLC.

In Fig.6, the obtained HPLC chromatograms are shown for the water-soluble portion treated with the wood charcoals prepared at various temperatures. Wood charcoals were clearly found to remove only the inhibitors from the water-soluble portion obtained by supercritical water treatment without reducing the levels of fermentable sugars. Wood charcoals prepared above 700°C showed high adsorption ability for the inhibitors in the water-soluble portion. From the fermentation tests of the treated water-soluble portions by wood charcoal, it was found that the treated water-soluble portions could be fermented to produce ethanol although ethanol was not produced in the untreated water-soluble portion. This indicates that the wood charcoal treatment is effective for improving the fermentability of the water-soluble portion obtained by supercritical water treatment of lignocellulosics. With *Saccharomyces cerevisiae*, sugars except for xylose in the water-soluble portions could be fermented within 6h at high ethanol yield (Fig. 7). However, with *Candida shehatae* or *Pachysolen tannophilus*, ethanol could not be produced so high in yield as in *Saccharomyces cerevisiae*, although both hexose and xylose can be fermented.
4. ORGANIC ACIDS AND METHANE PRODUCTION FROM LIGNOCELLULOSICS BY SUPERCRITICAL WATER TREATMENT

Lignocellulosics can be decomposed in supercritical water (>374°C, >22.1MPa) and converted to various useful products, such as saccharides and organic acids. Organic acids are appropriate substrates of methane production in anaerobic fermentation. Therefore, an understanding of organic acid production is important for the development of biomass conversion process in supercritical water to methane. In this study, thus, the production of organic acids from Japanese beech (*Fagus crenata*) was investigated using a batch-type system. Just for comparison, the isolated cell wall components and model compounds were also studied [12].

During a relatively short supercritical water treatment (380°C, 100MPa, 5sec), cellulose and hemi cellulose were decomposed to formic, pyruvic, glycolic, acetic and lactic acids, but there existed little in production of organic acids from lignin under these conditions. However, for more prolonged treatment (380°C, 100MPa, 4min), propyl side chains of phenylpropane unit of lignin was found to be decomposed to organic acids. Under these conditions, the most predominant organic acid recovered was acetic acid, probably derived from acetyl groups in the hemicellulose of Japanese beech.

The relationship between the density of water under various pressures/temperatures and yields of organic acids as treated in supercritical water at 380°C for 4min is shown in Fig. 8. The maximum yield of the total organic acids was obtained at 25MPa (0.43g/ml), over the critical pressure of water. The yield of formic acid was low under all conditions tested, but higher yields were obtained at lower treatment densities. Maximum yields of glycolic acid and lactic acid were obtained at 25MPa (0.43 g/ml), while the yield of acetic acid reached a maximum at the highest pressure tested (100MPa, 0.72 g/ml). These results suggest that the amounts of organic acids produced in supercritical water might be manipulated by controlling the density (pressure) of supercritical water.

To optimize the supercritical water treatment of lignocellulosics to organic acids, we will have to conduct a study about the influence of treatment pressure on decomposition behavior of lignocellulosics. Furthermore, we will have to study the producing pathway of organic acids from lignocellulosics in supercritical water. Presently known decomposition pathway of cellulose is shown in Fig. 9, as treated in supercritical water.
5. BIODIESEL FUEL PRODUCTION BY SUPERCritical ALCOHOL TREATMENT

Biodiesel fuel (BDF) is one of the most promising bioenergies, which can be produced from oils/fats through transesterification. A current commercial process for BDF production involves the use of alkali catalyst, followed by the removal of the catalyst and saponified products from free fatty acids (FFA). In addition, oils/fats should be water-free since the presence of water has negative effects on the production process. These cannot allow the low-quality raw materials, such as waste cooking oil and waste industrial oil, available for their efficient utilization.

Therefore, our research group has developed catalyst-free supercritical methanol methods; one-step method called as Saka Process (Fig. 10) and two-step method called as Saka-Dadan Process (Fig. 11) for BDF production. In these methods, low-quality oils/fats, which contain much amount of water and FFA, are available due to simultaneous conversion of FFA to methyl esters. In addition, it was found that the two-step method allowed more moderate reaction conditions than those of the one-step method, thus applicable to the commercial process.

The objective of this current work was to reveal the reaction behavior and mechanism in the two-step method by a kinetic study and in-situ observation through sapphire glasses. BDF evaluation was also carried out in terms of fuel specification standards. As a result of the in-situ observation, it was found that transesterification of rapeseed oil in supercritical methanol (one-step method) required higher reaction temperatures than 350°C to realize homogeneous (one-phase) reaction. On methyl esterification of fatty acid in supercritical methanol, however, its reaction system was always homogeneous. This is a reason why the two-step method can realize more moderate reaction conditions than those of the one-step method. Although hydrolysis of rapeseed oil in subcritical water always made a heterogeneous (two-phase) reaction system, its reactivity should be high due to the high ionic product of subcritical water.

On the other hand, results of BDF evaluation indicated that the two-step method could produce high-quality BDF which satisfies the fuel specification standards in Kyoto, EU and US. In addition, relationship between total glycerol/FFA contents and fuel properties such as pour point and viscosity became clear.

Further work on the two-step supercritical methanol method will be focused on fuel properties for a variety of oils/fats, such as soybean oil, sunflower oil and palm oil as well as their wastes, in terms of their fatty acid composition. Through these studies, the potential of various oil/fat raw materials will be clarified.
Transesterification in supercritical methanol

\[
\begin{align*}
\text{CH}_2\text{COOR}^1 & \quad \text{R}^1\text{COOCH}_3 & \quad \text{CH}_2\text{OH} \\
\text{CH}--\text{COOR}^2 + 3\text{CH}_3\text{OH} & \quad \rightarrow \quad \text{R}^2\text{COOCH}_3 + \quad \text{CH}_2\text{OH} \\
\text{CH}_2\text{--COOR}^3 & \quad \text{R}^3\text{COOCH}_3 & \quad \text{CH}_2\text{OH}
\end{align*}
\]

Triglyceride (TG) | Methanol | Fatty acid methyl esters | Glycerol (G)

Esterification in supercritical methanol

\[
\begin{align*}
\text{R}^1\text{COOH} + \text{CH}_3\text{OH} & \quad \rightarrow \quad \text{R}^1\text{COOCH}_3 + \quad \text{H}_2\text{O} \\
\text{Fatty acid} & \quad \text{Methanol} & \quad \text{Fatty acid methyl ester} & \quad \text{Water}
\end{align*}
\]

\(R^1, R^2, R^3, R'; \text{hydrocarbon groups}\)

**Fig. 10** Scheme of the one-step supercritical methanol method (Saka Process) [13]-[16]

Hydrolysis in subcritical water

\[
\begin{align*}
\text{CH}_2\text{COOR}^1 & \quad \text{R}^1\text{COOH} & \quad \text{CH}_2\text{OH} \\
\text{CH}--\text{COOR}^2 + 3\text{H}_2\text{O} & \quad \rightarrow \quad \text{R}^2\text{COOH} + \quad \text{CH}_2\text{OH} \\
\text{CH}_2\text{--COOR}^3 & \quad \text{R}^3\text{COOH} & \quad \text{CH}_2\text{OH}
\end{align*}
\]

Triglyceride (TG) | Water | Fatty acids | Glycerol (G)

Esterification in supercritical methanol

\[
\begin{align*}
\text{R}^1\text{COOH} + \text{CH}_3\text{OH} & \quad \rightarrow \quad \text{R}^1\text{COOCH}_3 + \quad \text{H}_2\text{O} \\
\text{Fatty acid} & \quad \text{Methanol} & \quad \text{Fatty acid methyl ester} & \quad \text{Water}
\end{align*}
\]

\(R^1, R^2, R^3, R'; \text{hydrocarbon groups}\)

**Fig. 11** Scheme of the two-step supercritical methanol method (Saka-Dadan Process) [17]-[18]
6. CONCLUDING REMARKS

Through these lines of research, the CO₂ zero-emission type bioenergy utilization system was proposed as described in Fig. 13 to use efficiently the by-products and wastes such as glycerol for some other biofuel production process: Among biofuels obtained by supercritical fluid technologies, biomethane is converted through the enzyme, methanemonooxygenase (MNO) into bio-methanol which is, then used as a solvent, to liquefy solid biomass to achieve the liquid biofuel. In addition, in the biodiesel production process, biomethanol can be used as a solvent to convert oils and fats into fatty acid methyl esters (biodiesel). Furthermore, a by-product of glycerol produced during its transesterification process may be used as a good substrate into biomethane. Through these joint utilization systems of the wastes and by-products, it will be feasible to establish the CO₂ zero-emission type bioenergy utilization system. Together with a depletion of fossil resources and deterioration of our earth environments, it will become more and more important to utilize the biomass resources. For such circumstances, we would like to propose such zero-emission type utilization system of bioenergies, and the biofuels developed in this work will substitute for fossil resources to mitigate the environmental loading.
7. REFERENCES


