Thermal Properties of Spent-Sawdust Matrix after Cultivation of *Grifola Frondosa* for Bioethanol Production

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***Abstract*-Maitake mushroom, which is the fruiting body of *Grifola frondosa* (white-rot fungus), is extensively cultivated in Japan using mainly hardwood-sawdust (HS) as the medium. After cultivation, a large amount of spent-sawdust matrices (SSM) is discharged, resulting in high disposal cost. It is therefore necessary to develop effective methods for the bioconversion of SSM into fuels and chemicals. The moisture content of SSM is approximately 70%, and hence it should be dried to prevent decay during storage and reduce the transportation cost. In the present study, the thermal properties of SSM and their effects on the enzymatic hydrolysis of SSM were investigated by carrying out a differential thermogravimetric analysis (TG/DTA). The thermal analyses showed that the thermal decomposition of SSM occurs more easily than HS. A high heating rate (100°C min^{-1}) and high temperature (500°C) did not improve the enzymatic hydrolysis of SSM. The optimal drying temperature for grinding and saccharification ranged from 25 to 200°C, while the optimal rate of temperature increase was 50°C min^{-1}. Under these conditions, the sample temperature of was approximately 121°C. The results of this investigation support our previous conclusion that an autoclave treatment of SSM at 121°C is effective in improving the enzymatic hydrolysis of SSM.**

Keywords- ***Thermal Properties; Thermal Analysis; Spent-Sawdust Matrix; Enzymatic Hydrolysis; Grifola Frondosa***

I. INTRODUCTION

Edible mushrooms, which are cultivated on media consisting of wood powder or chips, are very important food items in the world. Globally, more than 6 million tons of mushrooms are produced annually with an estimated value of over 14 billion U.S. Dollars [1]. Among such edible mushrooms, Maitake is the fruiting body of the white-rot fungus *Grifola frondosa* and is a popular edible mushroom in Japan, China, and the U.S.A [2].

In Japan, Maitake is extensively cultivated using sawdust matrix (SM). After harvesting the mushrooms, large quantities of spent-sawdust matrix (SSM) are disposed as agricultural wastes, resulting in environmental pollution or high disposal cost [3]. For example, a certain Japanese Maitake factory constantly discharges over 230 tons (wet weight) of SSM on a daily basis. Biorefinery process such as bioethanol production from lignocellulosic biomass has been attracted a lot of attention in the world [4]. However, utilization of lignocellulosic biomass has some problems such as their seasonal variations, scattered distribution, and high moisture contents resulted in the high cost of transportation and storage [5]. Moreover, the enzymatic digestibility of lignocelluloses is not easy because of the existences of lignin and their networks [6]. SSM, which mainly consists of hardwood-sawdust (HS), is a lignocellulosic biomass and a useful material in the production of fuels and chemicals. The advantages of SSM as a raw material in the production of fuels and other chemicals include low cost and perennial availability in large quantities. Moreover, SSM has a potential to be easily decomposed because it is denatured by *G. frondosa*, which is a white-rot fungus and has lignin degrading enzymes.

We have been working on the development of methods for the bioconversion of SSM to fuels and chemicals [7, 8]. However, it is difficult to transport and maintain because of its high moisture content (approximately 70%). Hence, SSM should be dried to prevent decay during storage and reduce the transportation cost. However, the thermal properties of SSM that can be useful for drying have not been reported so far.

For the utilization of biomass, it is necessary to know its chemical composition and thermal properties [9]. Thermal analysis is particularly effective for characterizing heterogeneous organic materials such as woody biomass because of its convenience and reproducibility. Negro et al. have reported that the different components of a lignocellulosic biomass have different thermal behaviors [10].

The present study investigates the thermal properties of SSM for its effective drying and the effects of thermal heating conditions on the grinding efficiency and enzymatic digestibility by a differential thermogravimetric analysis (TG/DTG).

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II. MATERIAL AND METHODS

A. Substrates and Enzymes

Fig. 1 shows a flowchart describing the cultivation of Maitake and the generation of SSM. HS mainly consists of beech wood. HS, SM, and SSM were supplied by Yukiguni Mitake Co., Niigata, Japan. SSM is typically available in blocks, and it was manually homogenized before use. The monomeric sugars and Klason lignin contents of HS and SSM are listed in Table 1.

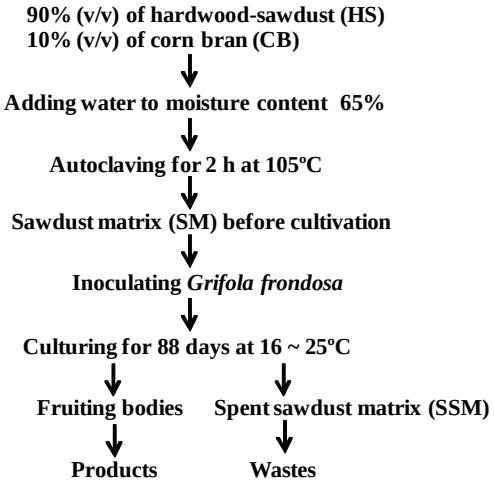

Fig. 1 Schematic diagram of the cultivation of *G. frondosa* and production of fruiting bodies

TABLE I MNOMERIC SUGARS AND LIGNIN CONTENTS OF HARDWOOD-SAWDUST AND SPENT-SAWDUST MATRIX

	Yields (%)	
Component	**Hardwood-Sawdust**	**Spent-Sawdust Matrix**
arabinose	0.7	0.8
rhamnose	0.4	0.3
galactose	1.1	0.9
glucose	36.9	37.1
xylose	16.9	14.9
mannose	1.2	1.5
Klason lignin	21.0	14.9

For the enzymatic hydrolysis of SSM, Cellulclast 1.5 L (cellulase derived from Trichoderma reesei QM9414) and Novozyme 188 (beta-glucosidase derived from Aspergillus niger) were supplied by Novozymes Japan Co. Ltd, Chiba, Japan.

B. Thermal Analysis of Raw and Freeze-Dried SSM under Normal Atmospheric Conditions

Thermal analyses of SSM were performed using the method described in our previous report [8]. Simultaneous thermogravimetry (TG), differential thermal analysis (DTA), and derivative thermogravimetry (DTG) of raw and freeze-dried SSM were performed using an EXSTRAR6000 TG/ DTA (Seiko Instruments Inc., Japan) at heating rates of 10°C min^{-1} and a temperature range from 25°C to 500°C under normal atmospheric conditions. The TG and DTG values were calculated using the following equations.

$$\text{TG (\%)} = (\text{weight loss due to thermal decomposition/ original weight}) \times 100 \quad (1)$$

$$\text{DTG (}\mu\text{g/min)} = \text{weight loss due to thermal decomposition/ time of heating} \quad (2)$$

DTA was simultaneously measured with TG on the TG/ DTA instrument, with α-alumina as the reference.

C. Effects of Different Conditions on Enzymatic Hydrolysis of Thermally Treated SSM

The raw SSM (approximately 30 mg, wet weight) was ejected from the thermal analysis equipment after its analysis within a temperature range of 25°C to 200°C or 500°C, and the rate of temperature increase varied between 10°C and 100°C min^{-1} under normal atmospheric conditions. The thermally treated sample was inserted into a micro test tube containing a stainless-steel bead and milled using a beads beater (TOMY Micro smash MS-100) at 4000 rpm for 30 s. The milled SSM was used as a substrate for enzymatic saccharification. The TG/DTA instrument was used for carrying out "thermal analysis" and "thermal dry treatment". One ml of the enzyme solution (Celluclast 1.5L: 0.8 filter paper unit ml^{-1} and Novozyme 188: 1.6 cellobiase unit ml^{-1}, in 0.05 M citrate buffer, pH4.8) was added to the micro test tube containing milled SSM and incubated at 50°C for 60 min. This was followed by centrifugation at 1100 ×*g* for 15 min. The amount of glucose in the supernatant was measured and

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the percentage glucose yield was calculated using the following equation.

$$\text{Glucose yield} = (B/A) \times 100 \quad (3)$$

Here, A is the glucose content in the SSM (mg) and B is the glucose obtained after enzymatic hydrolysis (mg).

D. Alkali Extraction and Hot Compressed Water (HCW) Extraction of SSM

Alkali and HCW extraction of SSM was performed using a previously reported method [7]. A 1% NaOH-solution was used as an indicator of the extent of wood decay by fungi. Approximately 50 mg of the sample was treated with 2.5 ml of the 1% NaOH solution for 1 h in boiling water. The treated samples were washed twice with 7.5 ml of hot water and 0.5 ml of 10% acetic acid solution. They were then dried at 105°C for 18 h. In the HCW extraction, approximately 50 mg of the sample was treated with 2.5 ml of water at 121°C for 20 min. The treated sample was segregated into water and solid residue, and then, the residue was dried at 105 ºC for 18 h. Alkali extraction, which is the solubility in 1% NaOH, and HCW extraction were calculated as the percentage reduction in the dry weight of the samples.

III. RESULTS AND DISCUSSION

A. Thermal Properties of SSM

Thermal analysis is a useful method for characterizing heterogeneous organic materials. The thermal behavior of organic materials depends on their chemical composition [10]. DTG and temperature curves of the sample and the reference cells under atmospheric conditions are shown in Fig. 2. The temperatures in the HS and SSM samples were lower than that in the reference cells. These differences can be mainly attributed to water evaporation and thermal decomposition. The DTG curves indicate the rate of weight loss by water evaporation and thermal decomposition. The DTG curves of HS and SSM show 3 main peaks: a low peak between 40 and 55 min, and 2 sharp peaks between 55-65 min and 80-82 min, respectively. In the previous research, the 3 peaks were respectively attributed to the decomposition of hemicellulose, cellulose, and lignin components. In this work, all the SSM peaks appeared faster than the HS peaks. The second and third peaks were relatively distinct. This suggests that the cellulose and lignin components of SSM were easily pyrolyzed in air as compared to those of HS. Yang et al. reported that the degradation and depolymerization of the recalcitrant bonds of lignin and cellulose through the biopretreatment of white-rot fungi can result in their easy pyrolization [11]. In other words, the cellulose and lignin components of SSM in our work were denatured during the long-term cultivation of *G. frondosa*, even though SSM and HS have similar sugar components, and the amount of lignin in SSM is slightly less than that in HS (Table 1).

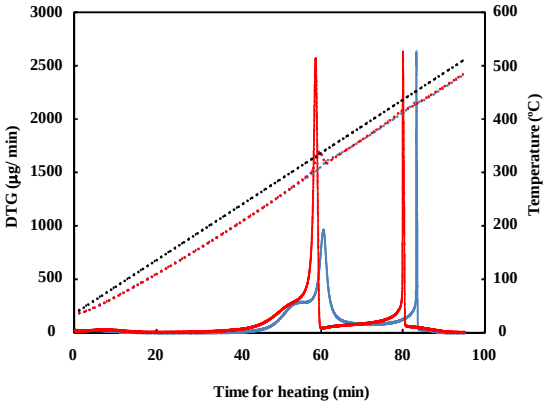

Fig. 2 Thermal analyses of HS and SSM under normal atmospheric conditions

The blue line is the DTG curves for HS, whereas the red line is the DTG curve for SSM. The red dotted line is the temperature for SSM, whereas the blue dotted line is the temperature curve for HS. The black dotted line represents the temperature curve for the reference cell.

The kinetic parameters were determined by the previous reports based on the Arrhenius equation [11, 12]. The activation energies of dried HS and dried SSM at 200-333°C were 112.04 kJ/mol and 69.29 kJ/mol, respectively. These results suggest that the long-term cultivation of *G. frondosa* can decrease the activation energy of thermal decomposition.

Thermal drying of SSM should be carried out under normal atmospheric conditions. The TG/ DTA curves of freeze-dried SSM (black lines) and raw SSM (red lines) under normal atmospheric conditions are plotted in Fig. 3. Weight loss in the freeze-dried SSM was observed at 220-340°C, and at 320-460°C, on the other hand, weight loss in raw SSM was observed at 100°C, 225-325°C, and 330-460°C. The considerable reduction in the weight of raw SSM at 100°C is attributed to the evaporation of free water in raw SSM. The DTA curve of the freeze-dried SSM suggests 2 significant exothermic reactions and no endothermic reaction. These exothermic reactions corresponded to the 2 sharp peaks in the DTG curves (Fig. 2). In the case of raw SSM, the DTA curve indicates one endothermic reaction (dotted square) and 2 large exothermic reactions; the

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endothermic reaction was observed at 25-100°C and is attributed to the evaporation of free water in raw SSM. The minimum DTA value was approximately 30 μV at 80°C.

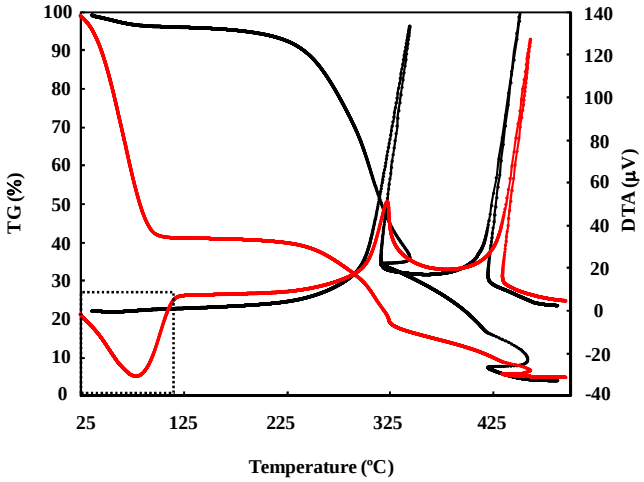

Fig. 3 Comparison of TG and DTA curves of freeze-dried SSM (black lines) and raw SSM (red lines) under normal atmospheric conditions
Dotted square showed DTA curve of raw SSM under 100ºC.

Fig. 4 shows the TG and DTG curves of SSM under different conditions and Table 2 lists these conditions in detail. At lower temperatures, the low heating rate resulted in weight loss. For example, there was a sharp decrease in the TG curve under condition (a) (10°C min^{-1}), but the TG curve decreased only slightly under condition (d) (100°C min^{-1}). In other words, a high temperature is required to achieve weight loss under Condition (d). The initial decrease in the TG curves was inhibited at approximately 120°C (a), 155°C (b), 222°C (c) and 260°C (d). These points correspond to those where almost all the free water in SSM had evaporated. Under a slow heating rate (10°C min^{-1}) a longer time was required for the complete evaporation of free water. The minimum DTA value gradually decreased with increasing heating rate (10-100°C). A high heating rate resulted in an increase in the endothermic reaction. Enzymatic hydrolysis was performed on samples at minimum DTA values under each condition (closed circles).

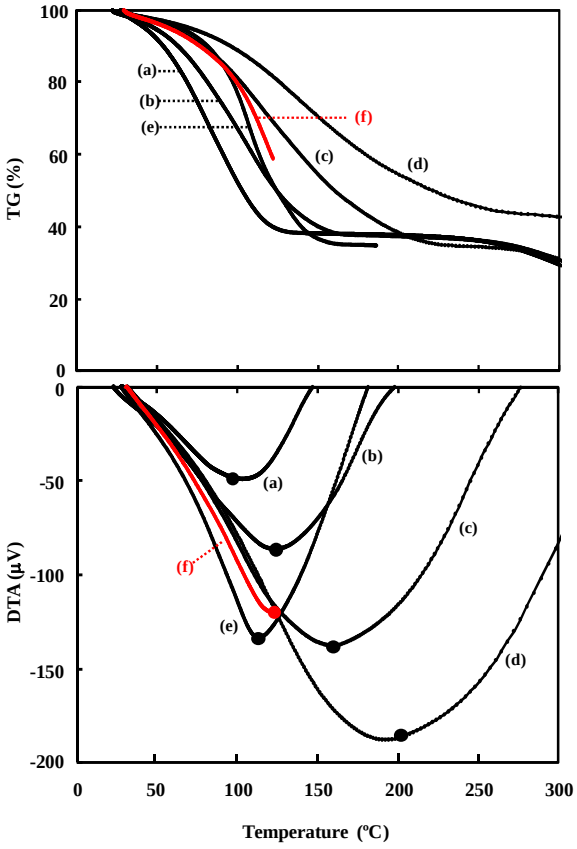

Fig. 4 TG and DTA curves under different conditions

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The letters correspond to the conditions listed in Table 2. The closed circles (black and red) denote the sampling points during the enzymatic hydrolysis.

TABLE II VARIOUS PARAMETERS OF THERMAL ANALYSIS IN DIFFERENT CONDITIONS

	Temperature range (°C)	Heating rate (°C/min)	Time to minimum DTA value (min)	Temperature of sample at minimum DTA value (°C)
(a)	25-500	10	12.21 ± 0.31	98.25 ± 2.72
(b)	25-500	20	8.85 ± 0.20	121.30 ± 4.38
(c)	25-500	50	5.53 ± 0.18	154.88 ± 10.28
(d)	25-500	100	4.14 ± 0.06	203.85 ± 5.51
(e)	25-200	50	5.41 ± 0.10	121.05 ± 1.76
(f)	25-200	100	4.30 ± 0.12	121.85 ± 1.97

B. Enzymatic Hydrolysis of Thermal-heated SSM under Different Conditions

Enzymatic hydrolyses of thermally dried SSM using the TG/DTA instrument are shown in Fig. 5. The glucose yields decreased with increasing heating rate ((a)-(d) and (e)-(f) in Fig. 5). At a high heating rate (100°C min^{-1}), some burnt deposits were observed on the surface of SSM even though a small amount of free water was present in SSM. The glucose yields were also affected by the preset temperature limit. The glucose yields in a preset temperature limit of 200°C were higher than that within a limit of 500°C. At a preset temperature limit of 500°C, the temperature of the sample increased significantly, leading to the formation of burnt deposits. On the other hand, the temperature of the sample increased gradually and almost no burnt deposits were observed within a preset temperature limit of 200°C. The sample temperature under condition (e) (50°C min^{-1}, 25- 200°C) was approximately 121°C (Table 2) whereas the sample temperature under conditions (c) (50°C min^{-1}, 25- 500°C) and (d)(100°C min^{-1}, 25- 500°C) were 155°C and 204°C, respectively; these temperatures were too high for carrying out sample drying (Table 2). These results suggest that a sample temperature of approximately 121°C is effective for the heat treatment of SSM samples for enzymatic hydrolysis. The thermal-treated raw SSM contained abundant free water, which is similar to the case of hydrothermal treatment with hot water. We have earlier reported that the glucose yield increases when the SSM is autoclaved at 121°C for 20 min before enzymatic hydrolysis; enzymatic hydrolysis for 24 h yields 36.0% glucose for raw SSM and 51.8% glucose for autoclaved SSM [7]. The present study, which is based on the thermal properties of SSM, confirms our previous report. In general, the optimum temperature for HCW treatment is 150- 200°C for the enzymatic hydrolysis of lignocelluloses such as that present in rice straw and eucalyptus [13-15].

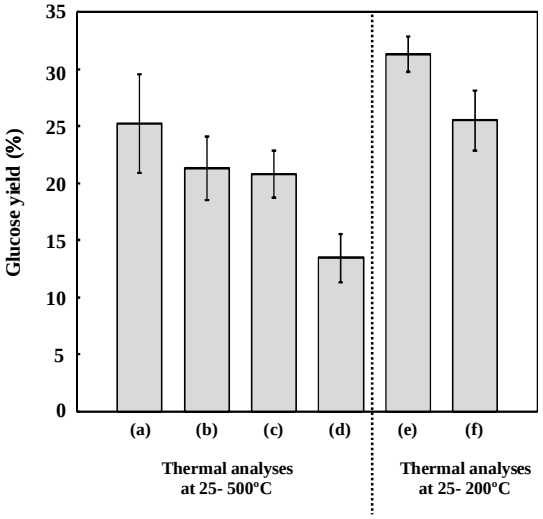

Fig. 5 Enzymatic hydrolyses of thermal-dried SSM under various conditions
The letters correspond to the conditions listed in Table 2.

The sample temperature of 121°C required for efficient enzymatic hydrolysis of SSM is lower than the sample temperature of 150-200°C required for HCW, because the SSM is denatured by the long-term cultivation of the white-rot fungus *G. frondosa*. Furthermore, alkali extraction is an indicator of the level of decay by the fungi and the results have shown that the amounts of alkali and HCW extraction (121°C, 20 min) of SSM were higher than that of HS (Fig. 6). These results suggest that SSM is fragile and can be easily decomposed by hot water at about 121°C.

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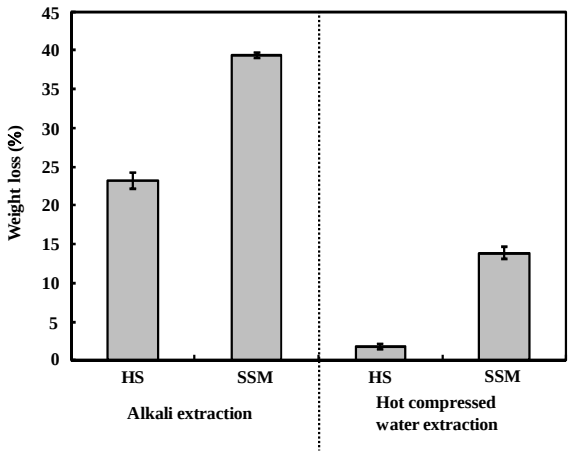

Fig. 6 Alkali extraction and HCW treatment of HS and SSM

IV. CONCLUSIONS

Thermal properties of SSM were investigated by using a TG/DTA instrument. It was found that the cellulose and lignin components of SSM were easily pyrolyzed in air as compared to those of HS, because SSM was denatured during the long-term cultivation of *G. frondosa*. Furthermore, the optimum drying temperature to enhance the enzymatic digestibility of SSM was found to be 121°C, which is lower than that of general hot-compressed water treatment (150-200°C).

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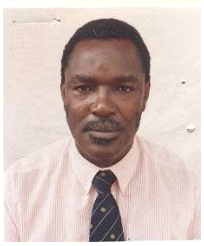

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