A Pilot Plant Scale 2nd Generation Bio-ethanol Production from Waste Mushroom Beds in Japan

Kouji YOSHIDA※1, Yosuke KOBAYASHI※1, Hiroto NISHIJIMA※1, Naohisa SUGIMOTO※1, Fuminori IMAI※2, Masatoshi KANEMATSU※2, Kenji YAMADA※2, Susumu ARAI※2, Kiyotaka SAGA※1† and Yoshiya IZUMI※1

(Received August 17, 2018)

The first pilot plant scale 2nd generation bioethanol production method from waste mushroom bed (or mushroom medium) with continuous steam explosion pretreatment has been documented. Waste mushroom bed is one of the few hopeful feedstocks for ethanol production in Japan. Steam explosion is known as an effective pretreatment for ethanol production from mushroom beds. The optimal condition for the continuous steam explosion was evaluated via fiber analysis and flask-sized saccharification. The exploded mushroom bed was then loaded into an 8 m³ saccharification vessel and saccharized with commercial cellulase for 48 h, then transferred over to a 5 m³ fermenter. The ethanol concentration level for the final product was 1.8 %. Conversion ratio for C6 sugars contained in the pretreated waste mushroom bed was 61 %.

The pilot plant scale demonstration of WMB-ethanol production was carried out with the aim of clarifying the problem which would occur at the commercial level plant for WMB-ethanol. Our pilot plant has been constructed in Kawasaki-City, Kanagawa, Japan to evaluate its ability to produce bio-ethanol from different types of biomass.

Steam explosion was chosen as cost and energy effective pretreatment. It also reported as effective pretreatment on WMB.

1. Introduction

Japan has 4 seasons, which brings sharp fluctuations of biomass supply. This fluctuation results in a difficulty in producing bio-ethanol due to the lack of biomass supply. Using waste mushroom beds (WMB) as one of the candidates for 2nd bio-ethanol resources has a benefit of constant supply. The annual abundance of WMB in Japan is estimated to be 257 Gg (257 kt)¹. This is enough to produce 60,000 m³ of bio-ethanol using the hypothesis that WMB contains 50 % of holocellulose and 80 % of holocellulose can be converted into bio-ethanol².

2. Experimental

2.1 Materials

The waste mushroom beds used in this study were purchased from farmers in Nagano Pref., Japan which was a byproduct of buna-shimeji (Hypsizygus marmoreus) cultivation.
2.2 Outline of our pilot plant:

Fig. 1 shows the outline of the pilot scale bio-ethanol plant. Raw biomass was carried to the Live Bottom Bin TM (Andritz Co. Ltd.) via the bucket lift which flipped over (Tumble Lift, Taiyu Co. Ltd.) and transferred the biomass to the Lock Hopper (double dumper) chute which could be done continuously. The biomass was then loaded into continuous steam explosion reactor (Steam EX™ Andritz Co. Ltd., Capacity: 2.5 t-BD/day). The loaded biomass sample was steamed at 180 to 200 °C within 8 to 22 min (hydrothermal reaction). Afterwards, the biomass treated in the hydrothermal reactor was transferred into the explosion vent (internal diameter: 10 or 15 mm) and then released into atmospheric pressure at the blow cyclone. Steam exploded biomass was separated from steam at the blow cyclone and dropped into the saccharification vessel (8 m³). Exploded samples could be collected from the sampling vent of the blow cyclone. Exploded biomass was suspended into the initial water which was prepared beforehand for the final solid content. The content was stirred via mixer driven by an inverted 7.5 kW agitator. The temperature of the saccharification vessel was controlled at 50°C. The process of saccharification started by adding cellulase. Steam explosion and saccharification was performed simultaneously. Therefore, the cooling tower water was supplied to the jacket during steam explosion to avoid overheating due to the heat of steamed biomass. Agitation and incubation were kept during the term in which saccharification was occurring. The saccharized biomass was then transferred to the fermenter (5 m³ × 2 vessels with chilled or hot water jacket and double turbine blade driven by 3.5 kW inverted agitator). Saccharification and fermentation samples were collected from sampling ports of each vessel. The culture made from seed yeast was prepared in the 1 m³ seed vessel and aerated (air flow rate: 1 m³/min), and subsequently inoculated into the fermenter.

2.3 The experiment procedure

Optimized steam explosion condition for WMB was determined, before the pilot plant scale demonstration. Continuous steam explosions were performed under the conditions shown in Table 1. WMB was loaded into the steam explosion reactor continuously and the exploded sample was collected for data. One bucket was used for one condition and then the same process was done with a different condition after all the treated WMB in the reactor ran out.

Collected samples were then evaluated by flask scale saccharification and fiber contents analysis which was mentioned in the analysis procedures.

2.4 Yeast seed culture

Yeast was prepared as follows; 200 mL of yeast preseed culture was inoculated in the seed culture medium containing 1 g/L of KH₂PO₄, 1 g/L of MgSO₄, 2 g/L of urea, 2 g/L of (NH₄)₂SO₄, 10 g/L of glucose and 10 g/L of corn steep liquor and 0.3 g/L of antifoam (PE-M). The working volume was 800 L, aeration was 20 m³/h, stirred by doubled turbine blade driven by 1kW agitator and cultivation term was 24 h.

| Material                | WMB          |
|-------------------------|--------------|
| Loading weight          | 150 kg-BD each bucket |
| Moisture content        | 57 % approx. |
| Explosion vent          | φ 10 mm      |
| Target temperature      | 180, 190, 200°C |
| Retention time          | 10, 20 min   |

Fig. 1 Outline of the pilot plant
2.5 Pilot plant scale demonstration

The pilot plant scale continuous steam explosion, saccharification and fermentation were carried out under the conditions shown in Table 2.

All of the used vessels were pre-sanitized by 100 °C steam for 1 h. The WMB (approx. 1.2 t BD) was carried to the steam explosion reactor continuously and dropped into the saccharification vessel (8 m³ capacity) carrying 1 m³ of initial water which contains 5 FPU of commercial cellulase. Water was added to adjust the final volume to 6 m³ after the steam explosion finished. After the addition of water, we measured the final solid concentration. The saccharification temperature was kept at 50 °C by hot water or cooling tower water via vessel jacket. Saccharification was carried out under moderate stirring (30 Hz, PV ratio: approx. 0.2 to 0.3) for 48 h. The pH of saccharification was kept at 5 by adding a solution of 25% NaOH. 3 m³ of the saccharified solution was transferred to each fermenter (5 m³ capacity) and 0.8 m³ of yeast seed culture was inoculated into it. The final working volume was 3.8 m³. Fermentation was performed for 24 h under moderate stirring (30 Hz).

Saccharification and fermentation samples were collected every 24 h, then measured by HPLC as mention above.

2.6 The analysis procedures:

The procedures for flask scale saccharifications were carried out as follows; the reacting mixtures of saccharification consisted of 40 g-BD of each WMB, 10 ml of 1mol/L sodium acetate buffer (pH5.0), 5 FPU of commercial cellulase diluted to 200 ml volume (biomass concentration: 20%) in a 500 ml polypropylene bottle. Saccharification was carried out at 50 °C with rotary shaking at 200 rpm.

The Fiber contents analysis was based on the NREL method 6). Bone dried 0.5 g of each WMB sample was mixed into 8 ml of 72 % (w/w) sulfuric acid and stirred for 1 h. Additional 300 ml of distilled water would be poured to dilute the sulfuric acid concentration to 4 %, then was kept in autoclave (121 °C) for 1 h, then neutralized with CaCO₃ and filtered for HPLC. Released sugars were measured as mentioned below.

Monosaccharides of each WMB sample were measured by Dionex™ HPLC system using CarboPac™ PA-1 column (4 × 250 mm, Thermo Fisher Scientific Co., Ltd.). The pump program and eluents settings followed Hirayama's method 5).

Ethanol concentration derived from fermentation were measured by Bio-Flow (BF-7D, Oji Scientific Instruments Co., Ltd.) Measurements of moisture or solid contents were carried out with moisture analyzer MB23 (Ohaus Corp.) by using approximately 10 g of each sample.

3. Results and Discussion

The fiber contents of untreated and steam exploded WMB are shown in Fig. 2. In the figure shows that the xylan contents decreased in the conditions 190 °C/20 min, 200 °C/10 min. and 200 °C/20 min. whereas the glucan content at 200 °C treatment increased. This glucose increasing may occur in proportion to the decreasing of the xylan content. Other components were considered to be lignin and ash, but were not measured in this study.

All pre-treated WMB were more saccharized than the untreated control sample. However, mannose was not detected in any saccharifications, despite mannan being detected in the fiber analysis. The pre-treatment condition 200 °C/10 min. had the highest sugar yield as well as the highest glucan conversion ratio. Considering this knowledge, we carried out a pilot plant scale demonstration with 200 °C /10 min. continuous steam explosion treatment.
3.1 Pilot plant scale demonstration:

Fig. 5 shows the result of fiber analysis of pre-treated WMB (shown as Fiber contents) and saccharification (see ‘monomerized polysaccharides’ column: released sugars were calculated to polysaccharides). Sugar yields from glucan and xylan were 67.1% and 56.5%, respectively, in pilot plant scale demonstration.

Fig. 6 shows the result of fermentation. Initial sugar
concentrations were calculated from the results of the saccharification. Produced ethanol concentration was 1.8%. This result meant that 88% of C6 sugar (galactose and glucose) was converted to ethanol within 24 h. The yeast used in this study was not able to ferment C5 sugars since xylose-fermenting recombinant yeast strains are constructed through genetic engineering\textsuperscript{6}. It will be possible to produce more ethanol from xylose with recombinant yeast due to the nature of WMB containing an abundant amount of xylose.

The saccharification efficiency mentioned above, however, was low in the pilot plant scale demonstration despite being in the same condition of the flask scale evaluation which had contrasting results. Contamination can be ruled out as it was not observed during the saccharification term. One of the possible causes of such low efficiency may be due to agitation efficiency and the power in which the mixer uses to mix the solution (data not shown). Further research is required to fill the gap between flask and pilot plant scale for saccharification.

Final ethanol yield from steam exploded WMB was 59%. There were few reports on WMB-ethanol production, using another kind of mushroom bed (Shiitake)\textsuperscript{3}. The report mentioned 70% of ethanol yield under the biomass loading condition similar to this study (20%, 22.8% in this demonstration). Higher biomass concentration affects adversely\textsuperscript{3}.

The second largest content of fiber in WMB was xylan but the yeast used in this study was not able to utilize xylose to break them down. We also considered using a gene modified (GM) fermentable strain of xylose\textsuperscript{7}. However, our pilot plant is not legally allowed to use GM microbes.

4. Conclusion

The conditions for continuous steam explosion has been optimized for WMB. Pilot plant scale ethanol production from WMB has been carried out and evaluated. The fermentation efficiency for C6 sugars was 88% and this yield was high enough. However, the saccharification efficiency requires further improvement.

Acknowledgment

This project is supported by the New Energy and Industrial Technology Development Organization.

References

1) Saga, K. \textit{et al.}, 26th Annual meeting of J. Jpn. Inst. Energy, 3-10-11, Aug. 1-2, 2017, Nagoya, Japan
2) Maurya, D. P.; Singla, A.; Negi, S., \textit{3 Biotech}, 5, 597-609 (2015)
3) Asada, C.; Sakawa, A.; Sasaki, C.; Nakamura, Y., \textit{Bioresource Technol.}, 102, 1052-1056 (2011)
4) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D., Determination of Structural Carbohydrates and Lignin in Biomass, NREL Laboratory Analytical Procedure, 2008
5) Hirayama, S.; Yamaguchi, M.; Izumi Y., \textit{CHROMATOGRAPHY}, 33(1), 35-39 (2012)
6) Matsushika, A.; Inoue, H.; Kodaki, T.; Sawayaama, S., \textit{Appl Microbiol Biotechnol.}, 84, 37-53 (2009)
7) Kobayashi, Y.; Sahara, T.; Ohgiya, Y; Fujimori, K. E., \textit{AMB Express}, 8, 139 (2018)