< Original Research Paper >

Improvement in the Productivity of Xylooligosaccharides from Waste Medium after Mushroom Cultivation by Hydrothermal Treatment with Suitable Pretreatment

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Abstract

The effective xylooligosaccharides (XOs) production from the waste medium after mushroom cultivation (WM) was investigated. The WM contains rich nutrients (protein, etc.) which induce Maillard reaction with reducing sugars under hydrothermal conditions. To improve the productivity of XOs, the suitable pretreatment combined with washing and grinding was investigated, and subsequently hydrothermal treatment was demonstrated with batch type and continuous flow type reactor. The washing pretreatment with hot water of 60 °C was effective to remove nutrients from the WM, and it led to prevent brownish discoloration on the hydrothermal treatment. On the basis of experimental data, industrial XOs production processes consisting of the pretreatment, hydrothermal treatment and purification step was designed. During the designed process, 2.3 kg-dry of the purified XOs was produced from 30 kg-wet of the WM (15 % yield as dry basis weight). Theoretical yield of XOs attained to 48 % as xylan weight in the WM.

Keywords: Xylooligosaccharide; Hemicellulose; Hydrothermal treatment; Pretreatment; Continuous flow reactor; Mushroom cultivation:

Abbreviations: XOs; xylooligosaccharides: WM; waste medium:

1. Introduction

Mushroom cultivation is one of the major agro-industry and it has become popular as healthy and functional food, and its production is also increasing. A type of food mushroom is cultivated artificially using the plastic pot that filled the medium material such as lignocellulosic biomass (e.g. sawn wood, corncob, wheat bran, rice bran, etc.). But after mushroom cultivation, most of the waste medium (WM) has not been utilized except for compost. Most of farmers want to utilize it for valuable product because of environmental and economical reasons.

It has been proposed utilizing the WM as resource of valuable products such as functional oligosaccharides, lignocellulosic composite materials, bioethanol, etc. (Amano, 2008). Hydrothermal treatment is one of the effective methods to fractionate the various components of lignocellulosic biomass (Parajó et al., 2004; Garrote et al., 1999; Sasaki et al., 2003; Goto et al., 2004) and to accelerate enzymatic degradation of lignocellulosic biomass (Yang et al., 2004; Liu et al., 2005; Wyman et al., 2005; Thomsen et al., 2006; and 2008). Makishima, *et al.* reported the hydrothermal treatment for the WM mainly consisting of corncob used for Enokitake (*Flammulina velutipes*) mushroom cultivation, was effective on xylooligosaccharides production from it. (Makishima et al. 2006). In addition, the xylooligosaccharides production from various agricultural biomasses using batch type hydrothermal reactor was reported by Garrote and Parajó (Garrote et al., 2001, 2002; Parajó et al., 2004; Vázquez et al., 2006). They analyzed the yields of xylooligosaccharides and derived products based on the kinetic modeling.

In a previous paper, the semi-pilot scale of continuous flow type hydrothermal reactor has been investigated to recover xylooligosaccharides from corncob, which is main component of the WM (Makishima et al. 2009). We obtained effective recovery of xylan fraction as mixture of xylose and xylooligosaccharides from corncob by using

tubular type hydrothermal reactor. However, the WM after mushroom cultivation contained rich nutrient (e.g. protein and amino acid) besides corncob. They induced Maillard reaction with reducing sugars under the hydrothermal conditions. As a result, the yield of sugars recovered from the WM decreased. Furthermore, brownish discoloration byproduct acted as significant impurities for the purification process of xylooligosaccharides.

In this paper, the effect of washing pretreatment to prevent brownish discoloration on the hydrothermal reaction for the WM has been investigated. Then continuous hydrothermal reaction for the pretreated WM was conducted with the semi-pilot scale of flow type reactor. Moreover, purification method for the crude xylooligosaccharide solution obtained from the WM has been developed. Finally, industrial production process of xylooligosaccharides from the WM has been designed, and its material balance was evaluated.

2. Materials and Methods

2.1. Raw material

The WM from Enokitake mushroom (*Flammulina velutipes*) cultivation after harvesting the fruits bodies was used as raw material for the hydrothermal treatment. Medium for cultivation of Enokitake mushroom is made of corncob, rice husk, rice bran, wheat bran, beet fiber and mineral components. The WM was supplied from the Agricultural Technology Institute of Nagano Farmer's Federation (Suzaka, Nagano, Japan).

2.2. Compositional analysis of the raw material

The WM composition was determined by standard analytical procedure for wood components as follows (Yamaguchi et al., 1986). The ash content was determined by

the weight loss after burning at 600˚C in the oven. The alcohol-benzene extract was determined by Soxhlet extraction method. Lignin content was determined using 72% sulfuric acid by the method of JIS P8008; 1976. Holocellulose content was determined using chlorite by the method of JIS P8012; 1976. α-Cellulose content was determined by the treatment with 17.5% NaOH solution. The protein content was estimated by semi-micro scale Kjeldahl method (protein = 6.25 × nitrogen).

2.3. Pretreatment of the raw material

In the preparation of raw material for the batch hydrothermal treatment, the WM was mixed with hot water to be the water content of 90 wt%. Then, it was washed by agitating for 30 min at different temperatures ranges from 40 to 100 ˚C. Slurry of washed WM was centrifuged at 1500 rpm for 5min, and removed the supernatant. The above washing step was continued twice. To reduce a particle size of the raw material, washed or unwashed WM were ground by using a wet grinder (MKCA 6-3, Masuko Sangyo Co., Ltd.). Washed and/or ground WM were dried in the drying oven at 60 ˚C for 6 hours, then it was stored in a desiccator at room temperature. These dried samples were subjected to the hydrothermal treatment with batch type reactor.

In the preparation of raw material for the continuous hydrothermal treatment, 30 kg of the WM (water content was about 58 wt%) was washed by mixing with 200 kg of hot water at temperature of 60 ± 10 ˚C and agitating for 30 min. Then, the slurry of washed WM was collected into a mesh filter bag (opening size is 0.84 to 1.00 mm) and squeezed out using a filter press under the pressure of 100 kg/cm^2. Particle size reductions of both raw materials which are washed or unwashed were conducted by using the wet grinder, and accordingly well-dispersed slurry of raw materials was prepared. These slurry raw materials were used promptly for the continuous hydrothermal treatment without storing.

2.4. Hydrothermal treatment of the WM with batch type reactor

The batch type reactor vessel made of SUS316 (Taiatsu Techno Co., Ltd., volume: 50 mL) was used in this study. The dried sample and distilled water 35 mL were filled in the reactor vessel. Different weights of dried samples from 1.5 to 6.7 g were used to be the slurry concentration range from 4.1 to 16 wt%. It was heated by an electric heater to control the reaction temperature. The experiments were carried out at reaction temperature ranges from 140 to 190˚C. Heat-up time was found to be about 20 min for all tested temperatures; thus 10 min of reaction time required a total 30 min. Cool down was achieved by soaking in water bath, internal temperature of reactor vessel was dropped to under 100 ˚C for 3 min.

After the hydrothermal treatment, the slurry of reaction product was fractionated by filtration to the solubilized fraction and residual fraction. The residual fraction was washed with distilled water and then dried in a drying oven. The solubilization ratio of sample was calculated from the weight of residual fraction.

2.5. Hydrothermal treatment of the WM with continuous flow type reactor

The tubular flow type reactor system was used in the continuous hydrothermal reaction. The detail of flow type reactor system has been described in previous report (Makishima, et al. 2009). This flow type reactor system was manufactured by Kimura Chemical Plants Co., Ltd (Amagasaki, Hyogo, Japan).

In the continuous hydrothermal treatment, the reactor system was warmed up to preset reaction conditions by feeding water. Then, the slurry material with and without pretreatment was fed by slurry pump to start the experimental run. Operation time was set as 0 min at this point. Fed slurry material was passed through heat-up divisions (H1, H2), reaction divisions (R1 to R4), cool-down divisions (C1, C2) and product discharge unit in order, and reaction product was discharged 25 min later. The hydrothermal

reactor system stabilized over 40 min after start experimental run, and then reaction products were collected. Reaction product was fractionated and solubilization ratio was calculated by same procedure described in the section 2.4.

The temperature of each reaction divisions (R1 to R4) were controlled to 185± 10˚C (in this case, 190˚C setting), and the residence time of slurry sample at the reaction divisions (i.e. reaction time) was controlled to about 10 min by handling the slurry flow rate within 300 to 325 g/min.

2.6. Sugar analysis of water soluble fraction from hydrothermal treatments

Total sugar content in the water soluble fraction obtained from the hydrothermal treatment was measured by the phenol-sulfuric acid method (Dubois, et al, 1956). The constituent sugars in the water soluble fraction were analyzed by high performance liquid chromatography (HPLC) equipment with refractive index detector. The sample solution was deionized using Amberlite MB-3 resin (ORGANO Co.) to remove various salts and filtrated with 0.45 μm membrane filter before HPLC analysis. The concentration of xylooligosaccharide was evaluated using the 1.0 mg/mL external standard. Xylooligosaccharide was analyzed by the HPLC conditions with MCIGEL CK02S column (20 mm I.D. × 250 mm, Mitsubishi Chemical Co.). The column temperature was controlled at 85˚C, and mobile phase was distilled water, pumped at 1.0 mL/min. To analyze the amount of constituent sugars, the HPLC conditions with Aminex HPX-87P column (7.8 mm I.D. × 300 mm, BioRad) was used. The column temperature was controlled at 85˚C, and mobile phase was distilled water, pumped at 0.6 mL/min.

3. Results and discussion

3.1. Effects of the washing pretreatment on the hydrothermal treatment of WM

Typical composition of the WM was as follows; α-cellulose, 22.9%; hemicellulose, 36.1%; Klason lignin,18.8%; ethanol–benzene extractives, 2.8%; ash, 10.0%; protein, 9.4%; on the basis for dried weight. These values had certain variation due to different lots of the WM. On the hydrothermal treatment for the WM, Maillard reaction occurred by combination of amino-compounds (e.g. protein) presented in the WM and reducing sugars produced from hemicellulose fraction. This brownish discoloration causes the difficulty to purify of xylooligosaccharide product, and it also causes yield loss of the product. In this section, the prevention of reaction to produce the brownish discoloration products was attempted by means of removing impurities (i.e. protein) with washing treatment for the WM.

To verify the effects of washing treatment, batch type hydrothermal treatment tests were conducted for the washed WM and the unwashed one. Reaction conditions were temperature ranges from 140 to 200 ˚C at the slurry concentration of 4.1 wt%. The WM washed at 100˚C was used in this test. As a result, light brown liquor was obtained in the 170 to 190 ˚C temperature range from the washed WM, in contrast to dark brown liquor obtained from the unwashed WM. It is indicated that brownish discoloration was prevented by washing treatment for the WM. Although weak coloration was also observed for the washed WM, it could be due to solubilization of lignin and production of furfurals derived by excess degradation of monomer sugars, and also Maillard reaction of remaining impurities.

Figure 1 shows total sugar content in the solubilized fractions obtained from each reaction condition. Solubilization ratio was decreased in the case of washed WM than the unwashed one (Figure 1a). It seems that easy-soluble fraction (e.g. sugars, proteins, amino acids, and mineral salts) in the WM was removed by washing treatment before hydrothermal treatment. Meanwhile total sugar content in the solubilized fraction was increased in the case of washed WM at over 180 ˚C than the unwashed one (Figure 1b).

At over 180 °C, the total sugar in the soluble fraction could be derived mainly from hemicellulose fraction of the lignocellulosic raw material. The increase of total sugars would result from the decrease of sugar loss caused by Maillard reaction in the hydrothermal treatment for the washed WM. On the one hand, total sugar obtained at under 170 °C would originate from easy-soluble fraction of the raw material. Total sugar content in the solubilized fraction was maximal at 190°C on the hydrothermal treatment for both washed WM and unwashed one.

Figure 2 shows the composition of mono- and oligo-saccharides detected by HPLC analysis for the solubilized fraction obtained at 190°C. Monosaccharides and sugar alcohols such as glucose, fructose and arabitol which were extracellular products by mushroom fungus were found in the solubilized fraction of unwashed WM (Makishima et al, 2006). Trace of trehalose and inositol, which were the intracellularly-stored sugars of mushroom fungus, were also detected. On the other hand, the contents of xylooligosaccharide, xylose and arabinose were increased in the case of washed WM. They were produced by hydrolyzing of hemicellulose fraction in the WM. Based on above results, it was indicated that the washing treatment for the WM could lead to improve yield of xylooligosaccharide production by the hydrothermal treatment.

3.2. Optimization of the washing treatment

Optimum washing conditions for the WM was investigated to minimize the expense of washing treatment. The WM was washed at temperature range from 40 to 100°C. Table 1 shows the compositional data of residual fraction (RF) and washable fraction (WF) on the washing treatment for the WM. About 26 wt% of unwashed WM (dry basis) was removed by washing treatment, but it was no significant change in the temperature range of 60 to 100°C. 60% of initial protein in the unwashed WM was removed as the WF, and protein content in the RF (i.e. “the washed WM”) decreased. It

is confirmed that protein in the WM was removed by the washing treatment, and it contributes to the yields of xylooligosaccharide production under the hydrothermal treatment for the washed WM.

Effect of the washing pretreatment on xylooligosaccharide production has been reported (Endo et al, 2000). They proposed the washing method with hot-compressed water of temperature ranges form 110 to 140 °C. By removing soluble lignin from xylan-containing biomass (e.g. corncob, bagasse) with hot-compressed water over 110 °C, they proposed excellent pretreatment method to prevent the coloration of crude xylooligosaccharide products obtained from the posterior hydrothermal treatment at 200 °C. But the WM contains excess amount of impunities like protein rather than soluble lignin, and it can be removed sufficiently with hot water under 60 °C. Therefore, it was concluded that optimum washing temperature was at 60 °C in the view point of energy consumption. The WM washed at 60 °C was used in the subsequent experiment in this report.

3.3. Effects of the material concentration on the hydrothermal treatment of waste medium with various pretreatment methods

To evaluate the production efficiency of xylooligosaccharide, hydrothermal treatment for the WM at various slurry concentrations were conducted with the batch type reactor. Hydrothermal treatment conditions were 190 °C and 10 min, and the concentration range from 4.1 to 16 wt% of solid raw material. Three types of the WM, which are ground and/or washed or untreated, were tested to verify the combination effect of pretreatment methods.

Figure 3 shows the yield of total sugars and solubilization ratio obtained from each WM. Solubilization ratio decreased slightly with the increase of material concentration for every type of WM as shown in Figure 3 a. It seems that the rate of solubilization for

WM could decrease with increasing the concentration of soluble fraction in the hot-compressed water at 190 °C. On the other hands, the solubilization ratio increased in the case of ground WM than the case of untreated one. Size reduction of solid raw material can lead to the increase of reaction efficiency, predictably. In the case of ground and washed WM, the solubilization ratio decreased because easy-soluble fraction in the untreated WM has been removed by washing treatment, like discussed in section 3.2. Meanwhile yield of total sugar obtained from the ground and washed WM was higher than other raw materials and it shows the advantage of washing treatment as shown in Figure 3 b. Total sugar yield was reached about 22 % as dried material weight within the tested material concentration range from 4 to 16 wt%. As described above, it is indicated that the raw material with high slurry concentration prepared from the combination of grinding and washing treatment contributes efficient recovery of xylooligosaccharide on the hydrothermal treatment for the WM.

3.4. Continuous hydrothermal treatment of the waste medium with flow type reactor

Continuous hydrothermal treatment for the optimally pretreated WM was demonstrated with the semi-pilot scale of flow type reactor as a step toward an industrial scale production. Based on above results, 12 wt% of slurry raw material prepared with the ground and washed WM was used in this test. Only ground WM was also used as control experiment.

With the ground and washed WM, the yield of total sugar was 24.8 wt%. Meanwhile, it was 18.3 wt% in the control experiment. The solubilization ratio obtained with the ground and washed WM was 48.2 wt%, it was comparable to the results of batch type reactor. It was confirmed that preparation of the optimally pretreated WM improves the yield of total sugar compared to the control test. In another experimental

run, stable continuous operation over 6 hrs was successful for the WM pretreated in the slurry concentration of 15.8 wt% or less (data was not shown) (Sato, et al., 2008).

3.5. Material balance on the xylooligosaccharide production process

Material balance on the xylooligosaccharide production using the hydrothermal treatment for the WM is summarized in Figure 4. It contains the overall process including the pretreatment, hydrothermal treatment and purification step. The values in Figure 4 mean the amount of products and byproducts in each step of the process. The values of purification process was also evaluated based on the experimental data (data was not shown). Detail of purification methods have been described in previous reports (Shinji, 2008; Makishima et al, 2009). Initial 30 kg-wet of the WM (15 kg-dry, water content was 50 wt%) was used to prepare the slurry raw material. Through the washing and grinding pretreatment, 3 kg of easy-washable fraction in the WM was removed as the component solubilized in waste water. Then 100 kg of slurry raw material (in the concentration of 12 wt %) was prepared for the hydrothermal treatment process. 4.8 kg of xylan, which is main component of corncob, was included in the prepared slurry. Following the hydrothermal treatment process, 80 kg of the solubilized fraction (i.e. crude xylooligosaccharide solution) and 20 kg of the solid residue (i.e. wet lignocellulose) were fractionated. The crude xylooligosaccharide solution contained total 3 kg of sugars consisting of xylooligosaccharide and monosaccharides such as xylose and arabinose. Furthermore, several purification procedures were applied for the crude xylooligosaccharide solution to obtain food grade products. First step was UF membrane filtration for decolorization, and second step was NF membrane treatment for elimination of monosaccharide and concentration of xylooligosaccharides. Third step was activated carbon treatment with controlling pH less than 4.0 for second decolorization. And then, ion exchange demineralization and second activated carbon

treatment was applied. Finally, 2.3 kg of purified xylooligosaccharide powder was obtained by the vacuum evaporation.

During the whole production process, 2.3 kg of purified xylooligosaccharide product was produced from 30 kg of the initial WM. And more specifically, its yield was 15 % of the initial WM as dry basis weight. Theoretical yield of xylooligosaccharide product can reach to 48 % of xylan weight in the initial WM.

4. Conclusion

Effective pretreatment on the hydrothermal treatment for the WM has been investigated. It can lead to prevent brownish discoloration on the hydrothermal treatment and it contributed to improve the yield of sugars in the result. Based on the obtained results, the continuous hydrothermal treatment with semi-pilot scale of flow type reactor and the purification obtaining food-grade XOs were demonstrated as a step toward industrial scale production. Following this success, we expect the commercial productions of XOs from biomass like the waste medium in local area.

Acknowledgements

This research was supported by Research and Development Program for New Bio-industry Initiatives (2004 - 2008) of Bio-oriented Technology Research Advancement Institution (BRAIN), Japan.

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Figure Captions

Fig. 1. Washing effects on a) solubility and b) total sugar for the hydrothermal treatment. The hydrothermal treatment were tested for waste medium without washing (white, circle) and waste medium washed at 100ºC (black, square).

Fig. 2. Constituent sugars in the solubilized fraction obtained by the hydrothermal treatment from the waste medium with or without washing (at 100ºC).

Fig. 3. Effects of slurry concentration on a) solubility and b) yield of sugars for the hydrothermal treatment of the waste medium with or without washing and/or grinding.

Fig. 4. Material balance of xylooligosaccharide production process with hydrothermal treatment for the waste medium.

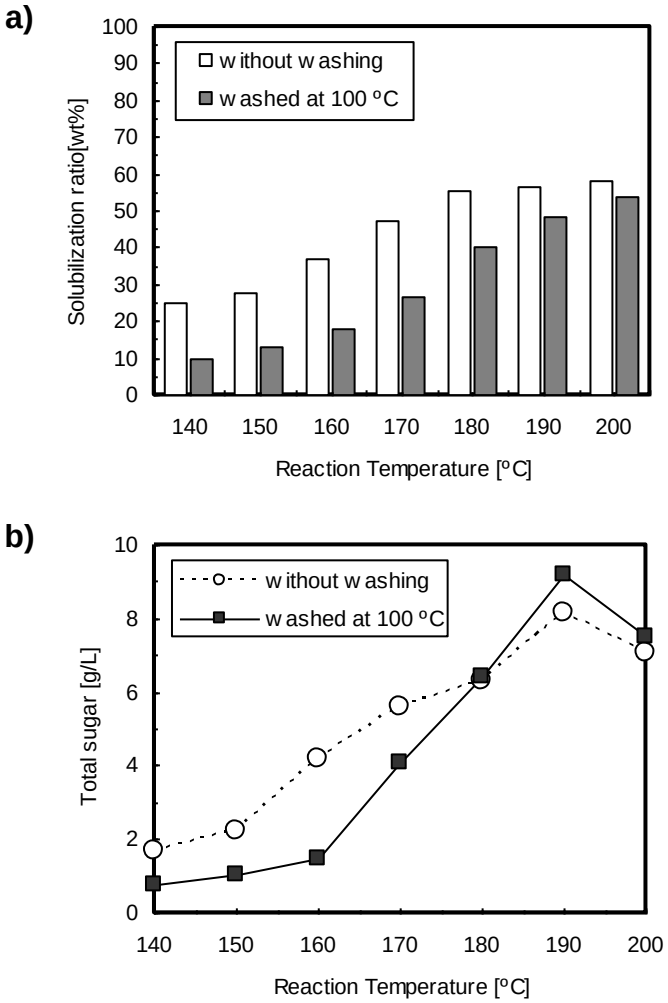

Fig. 1. Washing effects on a) solubility and b) total sugar for the hydrothermal treatment. The hydrothermal treatment were tested for waste medium without washing (white, circle) and waste medium washed at 100ºC (black, square).

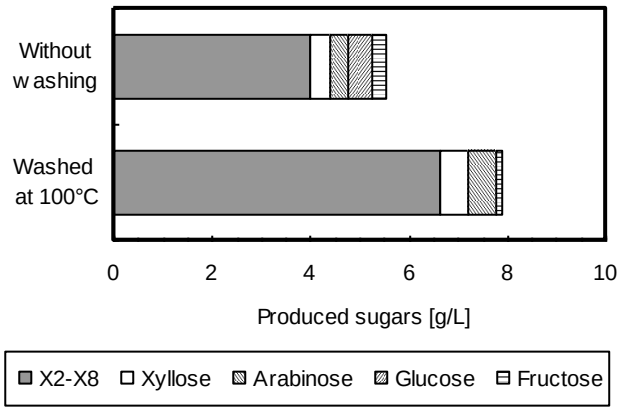

Fig. 2. Constituent sugars in the solubilized fraction obtained by the hydrothermal treatment from the waste medium with or without washing (at 100ºC).

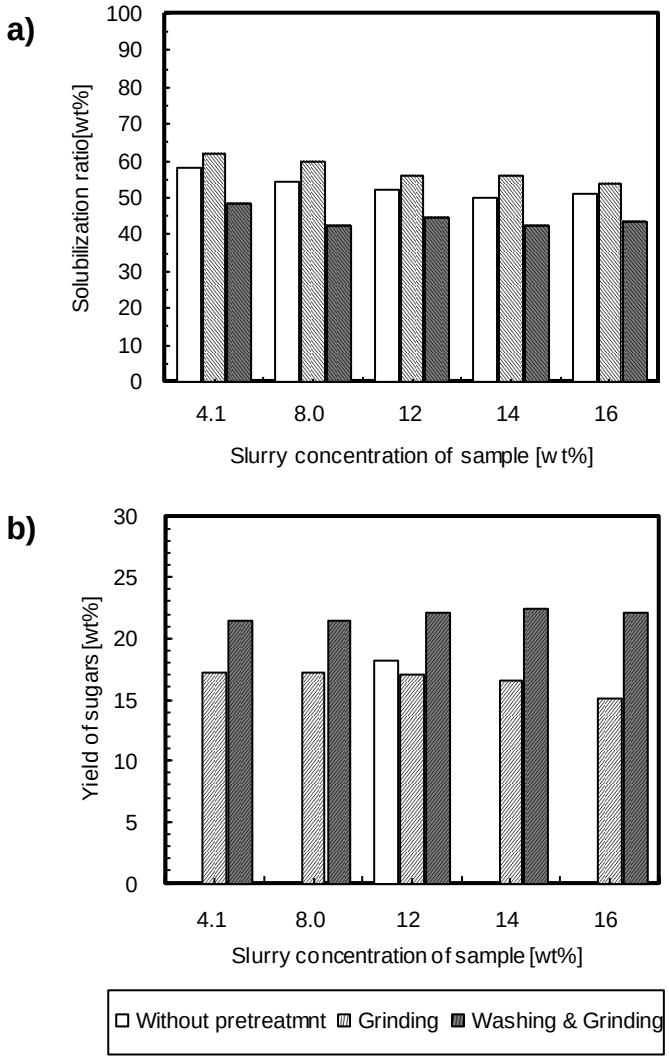

Fig.3 Effects of slurry concentration on a) solubility and b) yield of sugars for the hydrothermal treatment of the waste medium with or without washing and/or grinding.

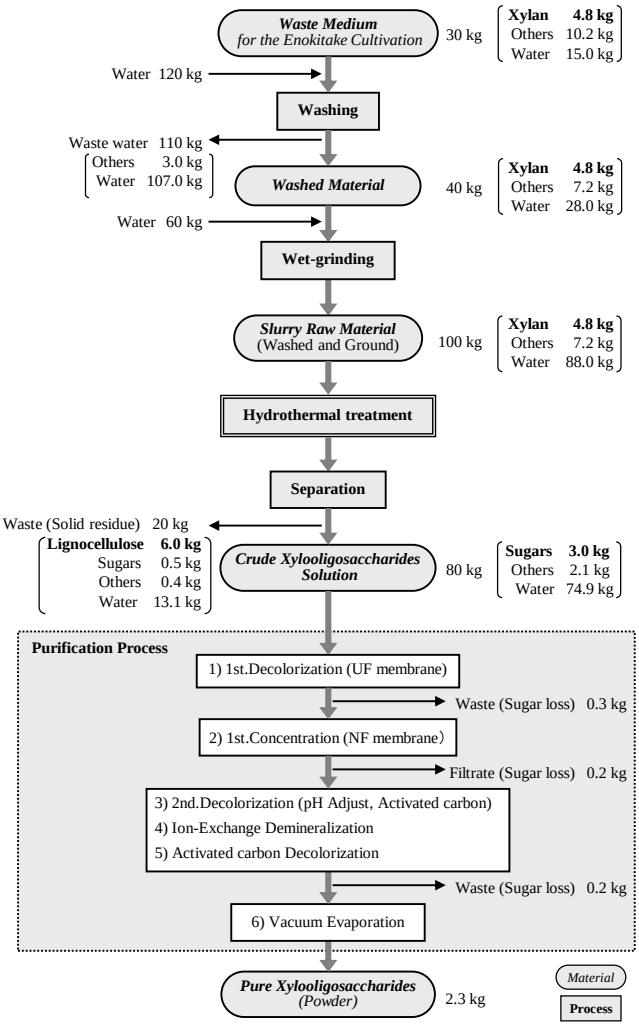

Fig. 4. Material balance of xylooligosaccharide production process with hydrothermal treatment for the waste medium.

Table 1. Components of washable fraction (WF) and residual fraction (RF) after washing at various temperatures.

Pretreatment	Residual fraction: RF				Washable fraction: WF				
	Subtotal of RF	Protein	Others [1]	Ash	Subtotal of WF	Protein	Sugars	Others [2]	Ash
Without washing	100	11.7	78.1	10.2	—	—	—	—	—
Washing at 40 ˚C	77.4	5.1 [a] *(6.6)* [*]	62.1 *(80.2)*	10.2 *(13.2)*	22.6	6.6	5.8	10.2	0.0 [b]
at 60 ˚C	73.5	4.6 [a] *(6.3)*	62.1 *(84.5)*	6.8 *(9.2)*	26.5	7.1	6.1	9.9	3.4 [b]
at 80 ˚C	73.4	4.5 [a] *(6.1)*	60.9 *(83.0)*	8.0 *(10.9)*	26.6	7.2	5.9	11.3	2.2 [b]
at 100 ˚C	73.7	4.4 [a] *(6.0)*	61.5 *(84.4)*	7.8 *(10.6)*	26.3	7.3	5.3	11.3	2.4 [b]

Unit: [wt%]

* *Values shown in parentheses are percentage in residual fraction.*

1) Unanalyzed fraction in residual fraction, such as cellulose, hemicellulose, lignin, crude fat, etc.

2) Unanalyzed fraction in washable fraction, such as organic acids, alcohols, etc.

a) By calculation as follows: [RF-Protein] = [Protein in the waste medium without washing] − [WF-Protein].

b) By calculation as follows: [WF-Ash] = [Ash in the waste medium without washing] − [RF-Ash].