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ORIGINAL PAPER

Microwave-Assisted Hydrolysis of Cotton Waste to Glucose in Combination with the Concentrated Sulfuric Acid Impregnation Method

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Abstract
Direct hydrolysis of a towel to glucose was investigated using microwave-assisted (MW) treatment combined with impregnation in concentrated sulfuric acid (SA) solution (51 wt% for 30 min) before the MW treatment. The maximum glucose yield by direct hydrolysis of an untreated towel was 30.9%, obtained at a heating temperature of 200 °C for 2 min. The maximum total glucose yield (from both direct hydrolysis and enzymatic hydrolysis of treated residue) was 74.2%, attained at a microwave heating temperature of 180 °C for 3 min. This condition is milder than that of MW treatment without impregnation in concentrated SA (our previous study, heating temperature of 200 °C for 7 min required). As shown by XRD of the unimpregnated and impregnated towels (in 9 and 36 wt% SA solution), the crystallinity index value decreased with the increase in SA concentration. Finally, 22.4 g of ethanol could be produced using the supernatant (containing glucose) and the microwave-treated residue as a carbon source for *Saccharomyces cerevisiae*, with less fermentation inhibition. Thus, impregnation in concentrated SA before MW treatment is effective for the production of glucose from cellulosic material and has low energy input.

Keywords Glucose · Hydrolysis · Microwave · Impregnation · Concentrated sulfuric acid · Ethanol fermentation

Statement of Novelty

This study developed a novel microwave-assisted hydrolysis method of cotton waste material combined with concentrated sulfuric acid impregnation (before the microwave-assisted hydrolysis) method. Compared with the conventional microwave-assisted hydrolysis methods in our previous study [1], this combination method proposed in this study has some advantages such as higher hydrolysis glucose recovery yield than that of microwave-assisted hydrolysis without impregnation in concentrated sulfuric acid and low input energy (lower treatment temperature and shorter treatment time). This combined hydrolysis method is expected to be effective in hydrolysis of glucose directly from cellulosic materials aiming for practical use in an industrial level.

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Introduction

Energy production from fossil fuels is harmful to the environment. Biochemical conversion of cellulosic biomass into bioethanol and other useful chemicals is one of the methods that has attracted worldwide attention.

In order to produce bioethanol and other chemicals via fermentation from cellulosic materials, it is necessary to hydrolyze the cellulose into fermentable sugars, especially glucose [2, 3]. However, since the cellulose molecules form intermolecular and intramolecular hydrogen linkages via their hydroxyl groups and exhibit a crystal structure, it is very difficult to hydrolyze them. Before the hydrolysis of cellulose, pretreatment such as mechanical comminution, chemical treatment, and hydrothermal treatment is necessary to reduce the cellulose crystallinity [4]. After the pretreatment, generally, many studies have used an enzymatic method to hydrolyze the cellulose. The enzymatic hydrolysis generally produces hydrolysates with a lower amount of substances that inhibit subsequent fermentation. However, the low specific activity of enzymes on cellulosic materials, requirement of some pretreatment steps, and the relatively

slower rate of hydrolysis make the acid hydrolysis method more competitive [5–7].

Recently, pretreatment and direct hydrolysis methods using supercritical, subcritical, and hot compressed water have been widely investigated and developed [8–12]. These methods have not only hydrolysis effect but also a pretreatment effect.

Microwave-assisted (MW) treatment is also a promising pretreatment and hydrothermal hydrolysis method. Although many studies using pretreatment (delignification) prior to enzymatic saccharification using the MW method have been reported in the past [13–15], recently, the direct conversion of cellulose model materials, cellulose in lignocellulosic materials, and starch into glucose and other valuable materials have been reported [16–19]. Furthermore, the MW method has been widely used for the production of oligosaccharides from curdlan [20] and hemicelluloses [21].

In our previous report [1], we studied the hydrolysis of cotton-based towels to glucose by MW treatment. Cotton-based waste (derived from the towel) is mainly composed of cellulose; therefore, it can serve as an alternative renewable biomass source for many valuable chemicals such as bioethanol without any influence by by-products derived from lignin and hemicellulose [22, 23]. The maximum amount of directly hydrolyzed glucose (28.9 g based on untreated dry towel of 100 g) was obtained at a MW heating temperature of 200 °C for 7 min with 1.0 wt% sulfuric acid (SA) as catalyst. Furthermore, the maximum amount of total glucose achieved from the towel, i.e., directly hydrolyzed glucose and enzyme-hydrolyzed glucose (residue after the MW was hydrolyzed by enzyme), was 78.0 g based on untreated dry towel of 100 g at a MW heating temperature of 200 °C for 7 min with 0.5 wt% SA as catalyst. Based on this result, in the next strategy, it is necessary to reduce the input energy for the hydrolysis and pretreatment of the towel sample using MW treatment.

In this study, to produce a high yield of glucose from the towel by the MW treatment, the towel sample was impregnated with concentrated SA solution before the MW treatment. In other studies [24–26], it was found that SA, at concentration more than 63 wt%, causes swelling and dissolution of cellulose samples. Swelling of the cellulose helps break the cellulosic crystallinity; subsequently, the chemical bonds, i.e., β-1,4-glycosidic bond in cellulose is degraded easily. Therefore, the amounts of directly hydrolyzed glucose obtained from towel samples impregnated with concentrated SA solution using MW treatment was investigated. Firstly, the concentration of the impregnation SA solution was optimized, and then, the MW treatment temperature and time were optimized. The treated solid residue was subjected to enzymatic hydrolysis and the total hydrolyzed glucose (via direct and enzymatic means) was evaluated; furthermore, the byproducts simultaneously generated with glucose, i.e., 5-hydroxymethylfurfural (5-HMF), levulinic acid, and formic acid (these were decomposed from glucose), from the towel were determined. Finally, the produced glucose (directly and enzymatically) was evaluated as a carbon source for the production of ethanol by *Saccharomyces cerevisiae*.

Materials and Methods

Materials

The towels used in this study were purchased from a local market in Tokushima and cut into small samples (2×2 cm^2). The cellulose content in the towel was 87.8%.

Direct Hydrolysis of Cellulose in Towel Samples to Glucose Using MW Treatment Combined with Concentrated Sulfuric Acid Impregnation

Before the treatment, the towel samples were ground (1 min, 5.0 g at one time treatment) using crush mill (D3 V-10, OSAKA CHEMICAL Co. Ltd., Osaka, Japan) to a mesh size of 500 μm.

The treatment consisted of two steps: decrystallization (impregnation) step and hydrolysis step. The overall experimental process schematic is represented in Fig. 1. The decrystallization step was carried out at room temperature for 30 min; 0.5 g of the towel samples were impregnated with 20 mL of 36, 51, or 55 wt% SA solution. After the impregnation, the samples were centrifuged and separated into solid and liquid phases. Forty milliliters of distilled water was added to the solid and centrifuged again. The supernatant was replaced with 20 mL of distilled water in a 20 mL reaction tube. The hydrolysis step was carried out using an Initiator$^+$instrument (Biotage Co. Ltd., Tokyo, Japan) at a frequency of 2.45 GHz, heated at 200 °C for 0.5, 1, 2, 3, and 5 min, and at 180 °C for 0.5, 1, 3, 5, and 7 min. After the reaction, the treated samples were cooled to room temperature and filtered.

Analysis of the Treated Towel Samples

Component analysis of the towel samples after the MW treatment was performed as follows: the solid (water-insoluble) and liquid (water-soluble) portions were separated by centrifuge; the solid portion was recovered, dried, and weighed. The cellulose content in the solid portion was determined on the basis of the monomer content (glucose) measured after the hydrolysis (72 wt%, H_2SO_4 followed by dilution). The hydrolysis method of the towel was applied to the Klason-lignin measurement method [27], i.e., the solid portion (1 g) was added to 15 mL of 72 wt% sulfuric

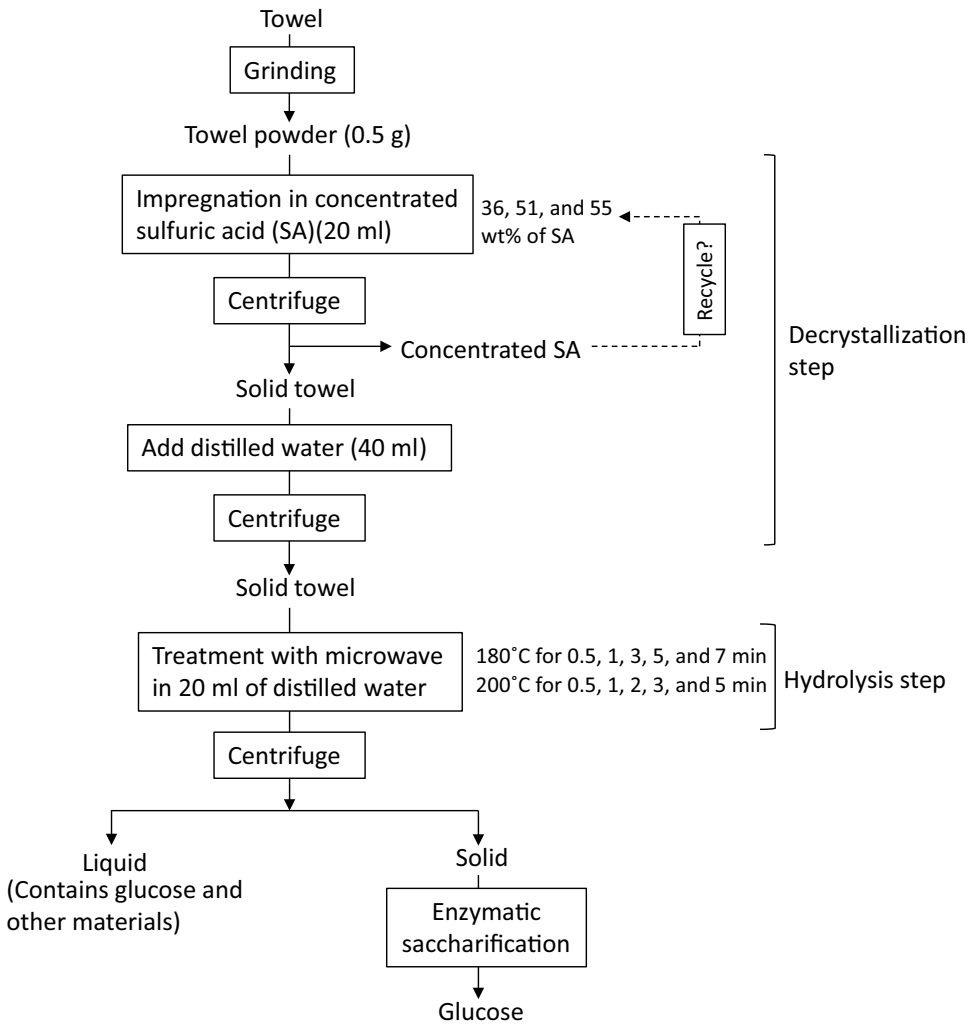

Fig. 1 Overall experimental process of the microwave-assisted hydrolysis of towel to glucose in combination with the concentrated SA impregnation method

acid and kept at room temperature for 4 h. After 4 h, the residue was placed in a 1-L conical flask, with 560 mL of distilled water, and then autoclaved for 1 h (121 °C). After cooling, the solid and the liquid portions were separated by centrifugation and the glucose in the liquid was analyzed by HPLC with a refractive index detector and a Bio-Rad HPX-87H column at 65 °C. The mobile phase used was 5.0 mM H_2SO_4 at a flow rate of 0.6 mL/min. Moreover, the glucose in the liquid portion (after the MW treatment) was determined using the mutarotase GOD method (Glucose C-II test, Wako Pure Chemicals Co. Ltd., Japan); other water soluble byproducts, namely, 5-HMF, formic acid, and levulinic acid in the water-soluble portion were analyzed with a HPLC system (the conditions as mentioned above). Other water-soluble components were determined by subtracting the amount of glucose and other byproducts from the water-soluble portion. All analytical determinations were performed in triplicate and the average of the results are shown.

Enzymatic Hydrolysis of the Water-Insoluble Portion of the Treated Samples

The water-insoluble portion of the treated samples was enzymatically hydrolyzed with the cellulase, Meicelase (derived from *Trichoderma viride*, 224 FPU/g β-glucosidase activity, 264 IU/g), which was purchased from Meijiseika-pharma Co. Ltd (Osaka, Japan). The enzymatic hydrolysis was performed using 10 mL of 0.1 M sodium acetate buffer (pH 5.0) at 50 °C in a rotary shaker, operating at 140 rpm, for 72 h. The substrate concentration and enzyme loadings were 20 g/L and 0.1 g/g of substrate, respectively. The supernatant was centrifuged to remove the solid residue and was analyzed for glucose. All the enzymatic hydrolysis experiments were performed in duplicate and the means were calculated. The glucose recovery yield by enzymatic hydrolysis (%) was calculated by the following equation:

(Amount of glucose produced (g)/Amount of cellulose in the treated towel sample (g) × 1.1) × 100.

Simultaneous Saccharification and Fermentation Using Direct Glucose and Pretreated Residue from the MW Method

Simultaneous saccharification and fermentation (SSF) was carried out using the enzyme Meicelase and *Saccharomyces cerevisiae* BA11 (Bio Academia Co. Ltd, Japan). The directly generated glucose and pretreated residue from the MW treatment (at a reaction temperature of 180 °C for 3 min) was used as the carbon source (before the MW treatment, the towel sample was impregnated with 51 wt% concentrated SA for 30 min). Moreover, the directly generated glucose and pretreated residue from the MW treatment (at a reaction temperature of 200 °C for 7 min with 0.5 wt% of SA as catalyst, without the SA impregnation) was used as a reference. *S. cerevisiae* BA11 is a relatively heat-tolerant yeast and it can ferment glucose to obtain ethanol at temperatures as high as 40 °C. This yeast was grown on potato dextrose agar plates at 37 °C and then stored in a refrigerator at 4 °C. A single colony of the yeast was added to 10-mL L-tubes containing 5 mL of sterile medium, which comprised of 20 g/L glucose, 10 g/L yeast extract, and 20 g/L polypeptone. All the chemicals used in this work were from Wako Pure Chemicals Industries (Osaka, Japan). This preculture was incubated at 40 °C for 12 h in a seesaw incubator at 60 rpm. The MW treatment was carried out for 5 times (0.5 g of towel could be treated at a time) and the samples were gathered. The supernatant (contained in directly hydrolyzed glucose) and the solid residue after the MW treatment were separated by centrifuge, and the supernatant was freeze dried, redissolved in distilled water, and then sterilized with an 0.22 µm filter. The solid residues were placed in 50 mL Erlenmeyer flaks and autoclaved for 20 min at 121 °C. Next, the sterilized supernatant, nutrient solution, enzyme, and sodium acetate buffer were added. The composition of the nutrient solution and enzyme loaded in the fermentation medium was adjusted by adding 10 g/L of yeast extract, 20 g/L of polypeptone, 0.1 g of enzyme/g substrate, and 0.2 M of sodium acetate buffer at pH 5.0. The precultured yeast suspension was centrifuged and the supernatant was removed, before the yeast was suspended in sterilized water and used to inoculate the fermentation medium, where its initial concentration was 0.25 g of dry cell/L in the mixture (40 mL). The mixture was incubated in a rotary shaker at 40 °C with gentle agitation at 100 rpm because *S. cerevisiae* BA11 can produce ethanol from glucose at this temperature.

Combined Severity Parameter

The logarithm of the combined severity parameter (log CS) was calculated using the pretreatment temperature T (in °C), pretreatment reaction time t (in min), and pH of the treated sample supernatant at room temperature in the following equation [28]:

$$\log CS = \log[H^+]t \exp\ ((T - 100)/14.75)$$

Determination of the Crystallinity Index (CrI) of Cellulose in the Towel Sample

The crystallinity index (CrI) of cellulose is defined as the percentage of crystalline part presented in the total cellulose. X-ray diffraction (XRD) is the most commonly used method to measure the CrI of dried cellulosic material. In this study, the CrI was calculated by peak height method, i.e., determining the height ratio between the intensity of the crystalline peak (I_{002}–I_{am}) and total intensity (I_{002}). The CrI value may not adequately explain the cellulose digestibility, because cellulose accessibility is not only affected by the CrI value, but it is also likely to be affected by particle size and porosity [29].

Untreated towel sample and treated samples (from decrystallization step) were analyzed using a X-ray diffractometer (Multiflex; RIGAKU, Yamanashi, Japan). The samples were scanned in the 2θ range of 5°–45° with the step size of 0.2° at 40 mA and 40 kV at a room temperature of 25 °C. The crystalline index (CrI) of untreated towel and treated samples were calculated from the X-ray diffraction patterns by the following equation [30–32]:

$$\text{CrI}\ (\%) = \left(I_{002} - I_{am}\right)/I_{002} \times 100$$

where I_{002} is the peak intensity from the (002) lattice plane (2θ = 22.6°) and I_{am} is the peak intensity of amorphous phases (2θ = 19.0°).

Results and Discussion

Effect of Impregnation with Concentrated Sulfuric Acid Combined with MW Method on the Direct Hydrolysis of Cellulose in the Towel Samples

In our previous study [1], we performed direct hydrolysis of cellulose present in a towel to glucose by the MW method. The maximum yield of glucose (28.9%) was obtained at a microwave heating temperature of 200 °C for 7 min with 1 wt% SA solution as a catalyst. Therefore, in the present study, direct hydrolysis of cellulose by this MW method was investigated using a towel made of waste cotton material (natural cotton or cellulose) as the biomass material. To obtain higher yield of directly hydrolyzed glucose from the towel with low input energy, the towel sample was impregnated with concentrated SA solution before the MW treatment. Generally, SA at concentrations greater than 63 wt% causes swelling and dissolution of cellulose samples [24–26]; in fact, 72 wt% SA is used for the Klason lignin

(high molecular lignin) determination in the lignocellulosic materials [27]. The 72 wt% SA plays a role in the swelling and dissolution of the polysaccharides in lignocellulosic materials (the method is described in our "Materials and Methods" Section). In our present study, the towel sample was impregnated with concentrated SA lower than 63 wt%, because the cellulose gets dissolved at higher SA concentration and it will be impossible to recycle the concentrated SA for the next cycle (after the impregnation, water should be added to the SA to complete the hydrolysis of the dissolved cellulose into monomer glucose). Therefore, we tested different SA concentrations of 55, 51, and 36 wt% as the impregnation solution (impregnation time was 30 min). Figure 2 shows that the directly hydrolyzed glucose yield from towel treated by MW combined with concentrated SA impregnation (based on dry untreated towel). The MW irradiation temperature and time were 180 °C and 200 °C for 7 min for the impregnated towel sample using 36 wt% SA; it was 180 °C for 3 min for the impregnated towel sample using 51 wt% SA and 180 °C for 1 min for the impregnated towel sample using 55 wt% SA. The result was compared with the directly hydrolyzed glucose yield from towel treated with MW without the SA impregnation. The maximum amount of glucose (29.2%) based on the dry untreated towel was observed at MW irradiation temperature and time of 180 °C and 3 min, which was similar to the glucose amount from towel without impregnation (28.9%). The pH values of the treated solution (supernatant) were 0.76 (impregnation) and 0.85 (without impregnation), respectively, and there was not much difference between them. However, there was no effect with the use of 36 wt% concentrated SA as impregnation solution, and in the case of 55 wt% SA the glucose amount obtained was lower (21.6%) than that of 51 wt%. The 55 wt% concentrated SA might slightly dissolve the cellulose sample. Furthermore, there were no changes in the glucose amount obtained with longer impregnation time (2 h).

CrI was indicated as one of the reasons for the increase in the glucose amount obtained by impregnation in concentrated SA before the MW treatment. Figure 3 shows the XRD result of the untreated towel, the towel impregnated with 9 wt% SA, and the towel impregnated with 36 wt% SA (impregnated samples were washed with distilled water only once and lyophilized). It was not possible to study the towel impregnated with 51 wt% SA because of the residual SA. The CrI value decreased with the increase in the concentration of SA, 0.71 for the untreated towel and 0.48 for the towel treated with 36 wt% SA. The 36 wt% SA solution facilitated the amorphization of the towel cellulose; however, it appeared that there was no effect on the subsequent hydrolysis by the MW. Therefore, 51 wt% SA might facilitate hydrolysis (without dissolving), besides amorphization.

From these results, we investigated the optimal condition to obtain directly hydrolyzed glucose and enzymatically hydrolyzed glucose from the residue after the MW treatment using the method combined with concentrated SA impregnation.

Effect of Various Microwave Irradiation Conditions on the Total Amount of Glucose and Other Chemicals Produced from the Impregnated Towel Samples

To evaluate the treatment conditions of the MW method for the production of glucose from towel impregnated with 51 wt% SA for 30 min, the following treatment variables were tested: reaction times (0.5, 1, 3, 5, and 7 min) under a reaction temperature of 180 °C, and reaction times (0.5, 1, 2, 3, and 5 min) under a reaction temperature of 200 °C.

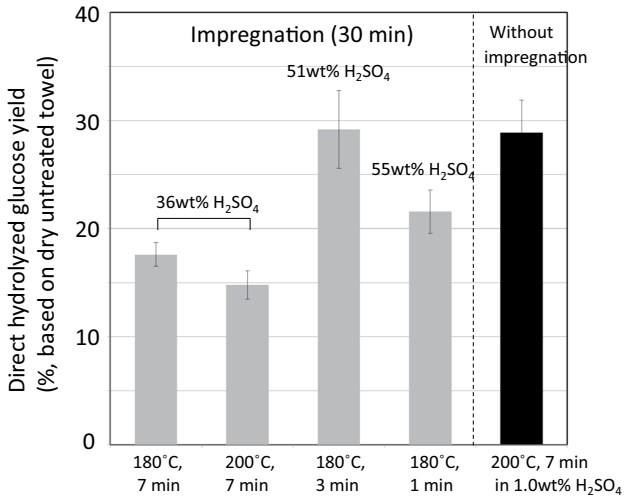

Fig. 2 Direct hydrolysed glucose yield from impregnated towel in concentrated SA

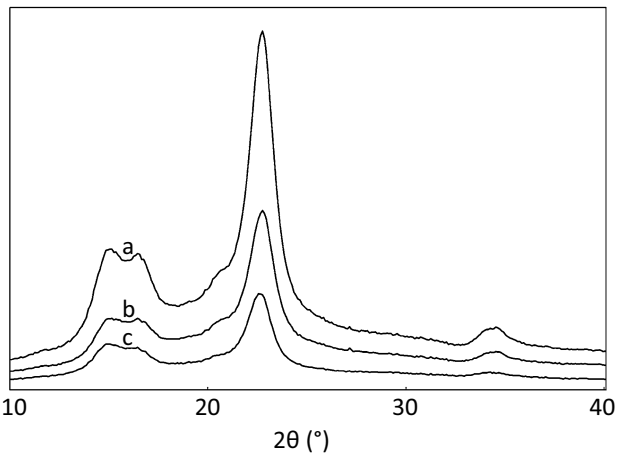

Fig. 3 X-ray diffraction spectra of **a** treated towel with 36 wt% of SA, **b** treated towel with 9 wt% of SA, and **c** untreated towel

To evaluate the total glucose amount hydrolyzed from the towel, the water insoluble portion after the MW treatment was hydrolyzed enzymatically. The total amount of glucose produced is summarized in Table 1. The maximum directly hydrolyzed glucose amount (30.9 g per 100 g based on untreated towel) was attained at the reaction temperature of 200 °C and reaction time of 2 min, and the next higher directly hydrolyzed glucose amount was 29.2 g per 100 g based on untreated towel at the reaction temperature of 180 °C and reaction time of 3 min. These conditions are milder than that of the condition with 200 °C for 7 min (in 1.0 wt% SA, without impregnation) and resulted in the highest direct glucose yield (28.9 g per 100 g based on untreated towel) without impregnation in SA solution before the MW treatment [1]; Therefore, impregnation before the MW treatment could reduce the energy input required to produce the glucose directly from the towel. Furthermore, with the total glucose, i.e., from the MW and from enzymatical treatments, the maximum glucose amount (74.2 g per 100 g based on untreated towel) was attained at the reaction temperature of 180 °C and reaction time of 3 min; the condition was also milder than that of the condition that resulted in similar maximum total glucose amount, 78.0 g per 100 g based on untreated towel, without impregnation before the MW treatment (the condition was 200 °C for 7 min, in 0.5 wt% SA) [1]. Overall, the SA impregnation before the MW treatment could reduce the energy input required to produce glucose from the towel sample.

To investigate the optimum condition for the production of glucose from the towel sample by the MW treatment method combined with the SA impregnation, the combined severity parameters (log CS) were calculated (Table 1). The analysis of the log CS showed that the highest total glucose yield (74.2 g per 100 g of untreated towel) was attained at log CS of 2.06 (180 °C for 3 min). However, the total glucose yield decreased with the increasing log CS values. This shows that the log CS value of 2.06 is the optimum MW condition (treatment time, temperature), for the method combining impregnation with 51 wt% SA solution before the MW treatment, that produces a maximum total glucose yield. On the other hand, log CS value at 200 °C for 7 min, in 0.5 wt% SA without impregnation before the MW treatment (highest total glucose yield, 78.0 g per 100 g of towel) was 2.57 [1].

Furthermore, to produce ethanol using the total produced glucose as the carbon source for the ethanol producing organisms, the degradation compounds other than glucose such as levulinic acid, formic acid, and 5-HMF were determined. These compounds can be used for a wide range of applications, such as a resource of polymers and plastics, resource of hydrogen, and important intermediates for the production of bio liquid fuel [33–36]. However, for the ethanol fermentation, especially using fungi (*Saccharomyces cerevisiae*), these compounds act as fermentation inhibitors [37, 38]. Fig 4 shows the glucose (directly and enzymatically) and other compounds yield based on the untreated towel. The degradation compound amounts from the condition of 180 °C for 3 min that shows the maximum glucose amount is 1/10 compared to the conditions of 200 °C for 7 min without impregnation. The conditions more severe than 180 °C for 3 min decreased the direct glucose amount, enzymatic glucose amount, and total glucose amount, especially, at 200 °C for 3 min and 5 min, and the degradation compounds increased. These compounds were generated by severe treatment conditions such as high temperature, long treatment time, and high acid or alkali concentration with heating [38–40].

Table 1 Glucose yields of microwave-assisted treated towel under various treatment conditions based on untreated dry towel of 100 g

Treatment temperature (°C)	Treatment time (min)	Combined severity parameter	Glucose by direct hydrolysis (g)	Water soluble components (except for glucose) (g)	Water insoluble portion (g)	Cellulose content in water insoluble portion (%)	Glucose recovery yield by enzymatic hydrolysis (%)*	Glucose by enzymatic hydrolysis (g)	Total glucose (g)
180	0.5	1.14	22.1 ± 0.9	3.8 ± 0.1	74.1 ± 1.3	71.7 ± 1.7	63.2 ± 5.3	37.0 ± 5.1	59.1 ± 5.1
	1	1.49	26.3 ± 1.0	2.2 ± 0.1	71.5 ± 4.5	69.1 ± 3.9	65.5 ± 4.9	35.6 ± 5.5	61.9 ± 5.5
	3	2.06	29.2 ± 3.6	1.9 ± 0.1	68.9 ± 5.5	70.7 ± 3.1	83.9 ± 3.0	45.0 ± 5.0	74.2 ± 5.0
	5	2.44	28.4 ± 1.6	5.6 ± 0.2	66.0 ± 1.8	65.0 ± 1.1	77.9 ± 5.8	36.8 ± 5.6	65.2 ± 5.6
	7	2.68	26.7 ± 2.2	12.6 ± 0.3	60.7 ± 2.0	70.2 ± 3.7	88.2 ± 4.3	41.3 ± 3.8	68.0 ± 3.8
200	0.5	1.78	25.8 ± 1.0	15.2 ± 0.3	59.0 ± 4.4	62.5 ± 5.0	62.2 ± 1.9	25.2 ± 5.2	51.0 ± 5.3
	1	2.02	27.7 ± 0.4	31.9 ± 1.0	40.4 ± 3.6	58.4 ± 5.0	83.6 ± 3.0	21.7 ± 4.4	49.4 ± 4.4
	2	2.58	30.9 ± 3.0	33.3 ± 1.1	35.8 ± 5.0	59.2 ± 1.0	94.4 ± 2.7	22.0 ± 4.9	52.9 ± 4.9
	3	2.83	23.6 ± 2.9	57.0 ± 2.3	19.4 ± 3.2	57.6 ± 0.1	73.3 ± 1.0	9.0 ± 3.5	32.6 ± 3.7
	5	3.18	14.5 ± 3.8	75.0 ± 5.7	10.5 ± 3.5	56.1 ± 2.0	57.7 ± 0.3	3.7 ± 3.6	18.2 ± 3.7

*Glucose recovery yield by enzymatic hydrolysis (%); based on cellulose in the treated towel sample (water insoluble portion)

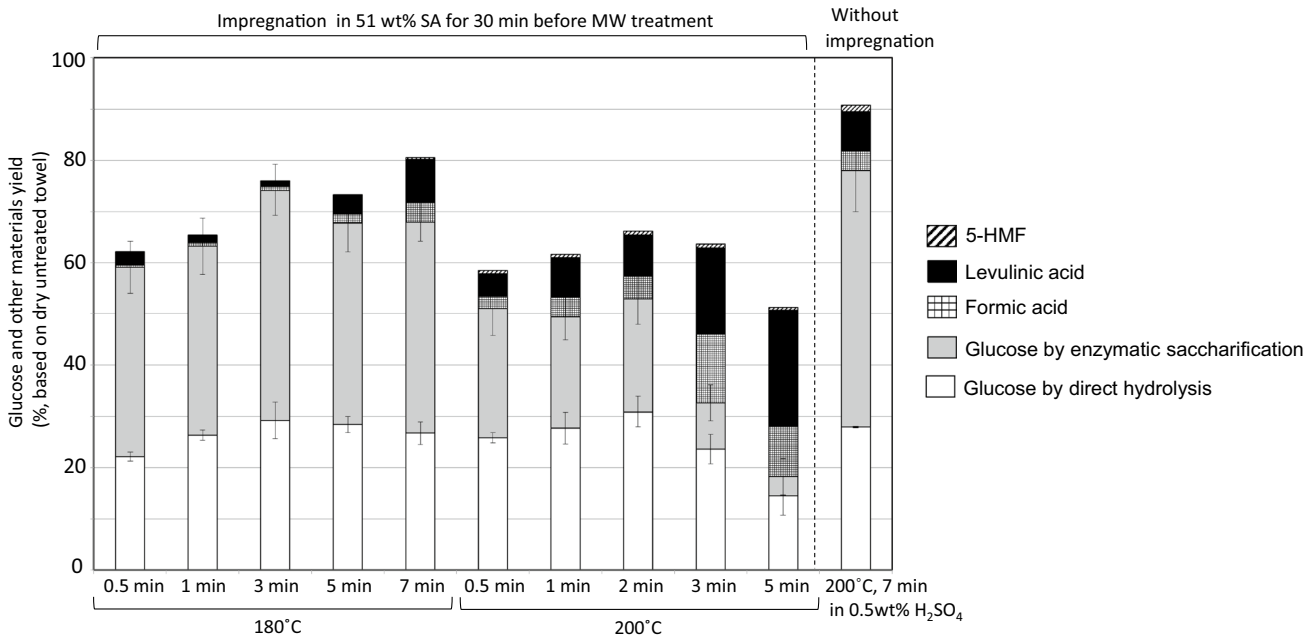

Fig. 4 Effect of treatment temperature and treatment time on yield of glucose and other water soluble materials from impregnated towel in 51 wt% of SA for 30 min before the MW treatment

Therefore, to use the glucose from this method as carbon source for ethanol fermentation using microorganisms, the condition of 180 °C for 3 min which resulted in more glucose and less degradation compounds is the optimum.

Ethanol Production using Glucose from Microwave Treated Towel

To evaluate the directly hydrolyzed glucose and pretreated residue as carbon sources for ethanol fermentation, the SSF experiment was carried out using *Saccharomyces cerevisiae* BA11. Ethanol production was investigated using directly hydrolyzed glucose (supernatant) and treated residue (water insoluble fraction) from towel impregnated with 51 wt% SA solution for 30 min and then microwave treated at reaction temperature of 180 °C for 3 min. Unimpregnated towel sample treated at 200 °C for 7 min with 0.5 wt% SA as catalyst was compared as reference (total glucose yield was 78.0 g per 100 g of untreated towel, Fig. 4). The ethanol production amounts (incubation time of 96 h) are summarized in Table 2; 22.4 g of ethanol from 100 g of untreated towel sample was produced from the impregnated towel sample. However, only 13.8 g of ethanol was produced from the unimpregnated towel sample. Overall, it was revealed that ethanol could be produced using the towel sample treated with MW in combination with 51 wt% SA impregnation, as a carbon source with low energy input.

Table 2 Ethanol yields (incubation times of 96 h) of microwave-assisted treated towel based on untreated dry towel of 100 g

	Impregnation in 51 wt% of SA solution for 30 min before the MW (180 °C, 3 min)	Only MW (200 °C, 7 min in 0.5 wt% of SA solution), without impregnation
Production amount of ethanol (g)	22.4 ± 3.8	13.2 ± 4.2

Conclusions

To decrease input energy for the direct hydrolysis of cellulosic materials, the effect of hydrolysis of towel sample by microwave treatment combined with concentrated SA impregnation was investigated. The combined method could directly produce a maximum of 30.9 g of glucose from 100 g of untreated (raw) towel, the towel sample was impregnated with 51 wt% SA solution for 30 min before the microwave treatment and then treated with microwave at temperature of 200 °C for 2 min. Total glucose, i.e. from directly and enzymatically, at an amount of 74.2 g from 100 g of untreated towel was achieved at the temperature of 180 °C for 3 min; subsequently, the glucose was converted into 22.4 g ethanol. It was found that this treatment condition could not only attain higher amount of glucose with low energy (5.6 Wh, power consumption of MW treatment at 180 °C, 3 min, on the other hand, in

previous study [1] power consumption was 10.3 Wh at 200 °C, 7 min), but also could decrease the degradation compounds produced along with glucose. Furthermore, the loading of cellulase (generally, 1/10 of the substrate) to hydrolyze the towel cellulose could be reduced (because the towel cellulose solid decreased after the MW treatment to be water insoluble portion). In future, this method could be tested with not only pure cellulosic materials, but also with plant biomass such as wood and straw. Furthermore, since the impregnation SA solution is not diluted, the possibility of recycling this solution or using safe solvent such as ionic liquid (and easier to handle than sulfuric acid) could be explored for further reduction in cost and energy.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest to declare.

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