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Saccharification behavior of cellulose acetate during enzymatic processing for microbial ethanol production

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HIGHLIGHTS

- The potential application of cellulose acetate to enzymatic processing.
- Prior to saccharification, cellulose acetate was subjected to deacetylation.
- After deacetylation, enzymatic saccharification at 50 °C yielded 88.1–99.1%.
- Cellulose acetate exhibited a temperature dependence during saccharification.
- Presaccharification prior to SSF was found effective for increasing ethanol yield.

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ABSTRACT

This study was conducted to realize the potential application of cellulose acetate to enzymatic processing, followed by microbial ethanol fermentation. To eliminate the effect of steric hindrance of acetyl groups on the action of cellulase, cellulose acetate was subjected to deacetylation in the presence of 1N sodium hydroxide and a mixture of methanol/acetone, yielding 88.8–98.6% at 5–20% substrate loadings during a 48 h saccharification at 50 °C. Ethanol fermentation using *Saccharomyces cerevisiae* attained a high yield of 92.3% from the initial glucose concentration of 44.2 g/L; however, a low saccharification yield was obtained at 35 °C, decreasing efficiency during simultaneous saccharification and fermentation (SSF). Presaccharification at 50 °C prior to SSF without increasing the total process time attained the ethanol titers of 19.8 g/L (5% substrate), 38.0 g/L (10% substrate), 55.9 g/L (15% substrate), and 70.9 g/L (20% substrate), which show a 12.0–16.2% improvement in ethanol yield.

1. Introduction

Cellulose acetate is one of the important cellulose derivatives for producing various consumer products including textiles, plastic films, and cigarette filters. The properties of cellulose acetate depend on its degree of substitution (DS), and cellulose diacetate with a DS of 2.5 is widely used because of its good solubility in solvents, molecular weight, and melt properties (Puls et al., 2011). Recently, how to reduce the tremendous amount of wastes generated from daily life and industry has become a serious problem of public concern. Since the global production of cellulose-acetate-based materials exceeds 800,000 tons per year (Puls et al., 2011), investigations on the biological utilization of cellulose acetate could prove useful for diminishing the environmental impact of these waste materials.

Although there is a question of whether cellulose acetate is susceptible to biological degradation, recent studies have led to a considerable increase in the knowledge of the biodegradability of cellulose acetate. Researchers have thus far isolated bacterial strains capable of degrading cellulose acetate with a DS of 1.7–2.5 (Moriyoshi et al., 2002; Ishigaki et al., 2000). Previous studies on the effect of DS on the biological utilization of cellulose acetate have been reviewed (Puls et al., 2011). The importance of deacetylation in the efficient biological degradation of cellulose acetate has been elucidated, in which acetyl esterases play a crucial role in the initial step and the resulting cellulose backbone is susceptible to cellulase action (Puls, 2004). Acetyl groups can also be eliminated by chemical hydrolysis using base catalysts. During chemical hydrolysis at room temperature, the acetyl content in cellulose decreases, and the glycosidic linkages are rather stable. The deacetylated cellulose with a DS of less than 0.8 is easily degraded by subsequent cellulase treatment (He et al., 2008).

Biorefinery is a concept that describes the production of fuels and chemicals from renewable resources via biological processes. Cellulose acetate is a renewable resource-based biopolymer produced from natural polysaccharides, and shows considerable potential to expand its applications to environmentally friendly

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materials (Mohanty et al., 2003). However, compared with the investigation on the degradation behavior of cellulose-acetate-based materials, few investigations have been reported on the production of fuels and chemicals through the biological utilization of cellulose acetate.

Microbial ethanol fermentation from saccharified cellulose has been studied extensively and is one of the approaches to assessing the availability of cellulosic materials as potential resources. In general, two ethanol fermentation processes have been proposed: separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). SHF, in which the activities of cellulase and microorganisms are inhibited by a high glucose concentration, requires a long overall process time. In SSF, the glucose produced during microbial fermentation can be converted rapidly into ethanol, thereby reducing process time (Alfani et al., 2000). Inevitably, however, there are also disadvantages of SSF over SHF; the optimum temperature in enzymatic saccharification is often higher than that in fermentation (Olofsson et al., 2008).

In this study, the potential use of cellulose acetate for biorefinery purpose was investigated through chemical deacetylation, enzymatic hydrolysis, and microbial ethanol fermentation. Particular attention is paid to the saccharification behavior of cellulose acetate depending on temperature and biomass concentration. The comparison of saccharification behavior and a strategy to increase process performance are also discussed.

2. Methods

2.1. Materials

Cellulose acetate was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The specifications of cellulose acetate given by the supplier are as follows: acetyl content, 38–40%; degree of substitution, 2.4; mean degree of polymerization, 150; and molecular weight, approximately 40,000. The lignocellulosic material used in this study was a water-insoluble fraction of rice straw obtained by hot water treatment, as described previously (Matano et al., 2012). Commercial cellulase, Cellic CTec2 (Novozymes, Bagsvaerd, Denmark) with a filter paper unit (FPU) of 120 per 1 ml (measured in our laboratory), was used for enzymatic saccharification. All other chemicals were of analytical grade. For microbial fermentation, the yeast *Saccharomyces cerevisiae* NBRC1440 (Matano et al., 2012) was used for ethanol fermentation.

2.2. Deacetylation of cellulose acetate

Cellulose acetate (1 g) was immersed in 20 ml of methanol/acetone (1:1, v/v), into which 1 ml of 1 N sodium hydroxide was added. The mixture was then allowed to stand at room temperature for 2 h. The cellulose acetate collected was washed thoroughly with distilled water and 50 mM citric acid buffer (pH 5.0), dried at 70 °C for 24 h, and then ground into powder prior to use. The weight loss of the sample was calculated as the difference in weight before and after deacetylation (He et al., 2008).

The glucose content in cellulose acetate was determined by sulfuric acid hydrolysis as described previously (Sluiter et al., 2008). The resultant sugar solutions were analyzed by high-performance liquid chromatography (HPLC) as described in the next section. The acetyl content in cellulose acetate was determined by the Eberstadt method (Genung and Mallatt, 1941). One gram of dry cellulose acetate was added to 40 ml of 75% ethanol, heated at 60 °C for 30 min, and saponified with 40 ml of 0.5N sodium hydroxide by heating at 60 °C for 15 min. The mixture was allowed to stand at room temperature for 48 h. An excess amount of 0.5N hydrochloric acid was then added to the saponified solution. The excess hydrochloric acid was titrated with 0.5 N sodium hydroxide to pH 7.0. A parallel blank experiment using crystalline cellulose was also carried out and acetyl content was calculated as follows:

$$\text{Acetyl content } (\%) = \{(A - B)Nb - (C - D)Na\} \times 4.3/W$$

where A: volume (ml) of 0.5N NaOH added to the sample; B: volume (ml) of 0.5N NaOH added to the blank; C: volume (ml) of 0.5N HCl added to the sample; D: volume (ml) of 0.5N HCl added to the blank; Na: normality of hydrochloric acid; Nb: normality of sodium hydroxide; W: dry weight (g) of the sample.

Changes in the chemical bonds and molecular structure of cellulose acetate were analyzed using a Fourier transform infrared (FT-IR) spectrometer (IRAffinity-1, Shimadzu Corp., Kyoto, Japan) equipped with an attenuated total reflection system. Spectra were obtained from an average of 20 scans from 700 to 4000 cm^{-1} with 8 cm^{-1} resolution.

2.3. Enzymatic hydrolysis

Cellulose acetate samples (5–20% loadings based on dry weight) were suspended in 50 mM citric acid buffer (pH 5.0). Enzymatic hydrolysis was initiated by adding cellulose to the samples at a dosage of 20 FPU/g cellulose acetate (unless otherwise noted). 50 ml polypropylene tubes (Corning Inc., NY, USA) containing the above solutions were incubated at 35 or 50 °C in a rotator (Thermo Block Rotator SN-06BN; Nissin, Tokyo, Japan). The sugar solutions obtained at specified intervals were analyzed by HPLC using an ULTRON PS-80H column (300 × 8.0 mm I.D.; Shinwa Chemical Industries Ltd., Kyoto, Japan) maintained at 80 °C. The mobile phase (pH 2.1) was prepared by adding perchloric acid to distilled water, which was then flowed at a rate of 0.7 ml/min. The absorbance of glucose was measured on a refractive index detector.

2.4. Ethanol fermentation

Microbial ethanol fermentation was carried out by SHF, SSF, or a combination of presaccharification and SSF. The yeast *S. cerevisiae* was aerobically cultivated at 30 °C for 24 h using a basal medium containing 10 g/L yeast extract, 20 g/L polypeptone, and 20 g/L dextrose (YPD). The cells were collected by centrifugation for 10 min at 6000*g* and washed with distilled water. For SHF, 5 ml of YPD containing 1 g-wet weight of *S. cerevisiae* was added to a 100 ml closed bottle containing 45 ml of the sugar solutions obtained by enzymatic hydrolysis at 50 °C for 48 h. The bottles were equipped with a bubbling CO_2 outlet and incubated at 35 °C (within the acceptable limits for ethanol fermentation using the yeast *S. cerevisiae* NBRC1440) at a stirring rate of 300 rpm. For SSF, cells were added into the mixtures of cellulose acetate in a closed bottle immediately after adding cellulase. Unless otherwise noted, the sugar solutions obtained by presaccharification at 50 °C for 24 h were used for ethanol fermentation. Ethanol and glucose concentrations in the fermentation broth were determined by HPLC under the same conditions as those described above.

3. Results and discussion

3.1. Deacetylation of cellulose acetate

Table 1 shows the glucose and acetyl contents in cellulose acetate before and after deacetylation by alkaline treatment. The acetyl content in cellulose acetate decreased significantly after deacetylation, in which the corresponding weight loss of cellulose acetate was observed. Accordingly, the glucose content in the deacetylated cellulose increased to 92.3 wt%. To further confirm