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Direct saccharification and ethanol fermentation of cello-oligosaccharides with recombinant yeast

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ABSTRACT

Ethanol was produced at good rates by direct saccharification and fermentation of cello-oligosaccharides with pYBGA1 yeast, a recombinant laboratory yeast expressing β-glucosidase. Cellobiose in the concentration of 50 g/L was directly fermented for 60 h with 1×10^8 cells/mL of pYBGA1 yeast at 30 °C to give ethanol at an 80% theoretical conversion rate and a concentration of more than 20 g/L of concentration. Conversion to ethanol increased with increasing cellobiose concentration in the feed. When cellobiose was used at the concentration of 100 g/L, ethanol conversion and concentration increased to 85% and 45 g/L, respectively, in 96 h incubation. Other cello-oligosaccharides, cellotriose, cellotetraose, and cellopentaose at the concentration of 50 g/L, respectively, were also fermented directly for 72 h with 1×10^8 cells/mL of pYBGA1 yeast to produce ethanol in the conversion rates and concentrations of 71–73% and 18.0–18.5 g/L, respectively. The direct saccharification and fermentation mechanism of cello-oligosaccharides with pYBGA1 yeast, as revealed by HPLC measurements, suggesting that cellotetraose, for example, was saccharificated to cellotriose, cellobiose, and glucose and then fermented to give ethanol. These results suggest that the direct saccharification and fermentation of cello-oligosaccharides with pYBGA1 has several advantages as a simple procedure and for time, cost, and energy consumptions.

1. Introduction

Cellulosic bioethanol from renewable plant resources is an environment-friendly and alternative energy to replace petroleum-based fuels because cellulose is the most abundant non-food resource in nature (Cardona & Sánchez, 2007; Wyman, 1996). However, unlike starch, there are several problems in producing ethanol directly from a cellulosic biomass. The lignin that comprises 30–40%of the lignocellulosic biomass must be removed because lignin inhibits enzymatic saccharification and fermentation. In addition, cellulose crystal regions in the molecule resist enzymatic saccharification. Furthermore, the utilization of hemicellulose, which constitutes 20–30%of the lignocellulosic biomass, is necessary for the economical production of ethanol. Therefore, pretreatment of the lignocellulosic biomass before saccharification of cellulose and fermentation is necessary. In the current industrial production of cellulosic bioethanol (Alvira, Tomas-Pejo, Ballesteros, & Negro, 2010; Sun & Cheng, 2002), hydrolysis of cellulose including the crystalline region to glucose by sulfuric acid is the only method to give ethanol in good conversion rates (El-Zawawy, Ibrahim, Abdel-Fattah, Soliman, & Mahmoud, 2011; Su, Tzeng, & Shyu, 2011; Sun & Cheng, 2005). However, the sulfuric acid method has several problems: a large amount of sulfuric acid is necessary, it is difficult to recover acidic wastewater, and the manufacturing plant readily corrodes under acidic conditions. Therefore, there are many reports on the production of cellulosic bioethanol by alkaline pretreatment to remove lignin and decrease the crystallinity of cellulose molecules and then simultaneous saccharification and fermentation with a combination of cellulase and yeast (Linde, Galbe, & Zacchi, 2007; Wu, Arakane, et al. 2011a; Wu, Li, et al., 2011b).

Several reports on the direct saccharification and fermentation of cellulose to produce ethanol have appeared. Ingram reported the conversion of waste office paper to ethanol by a recombinant strain *Klebsiella oxytoca*, in which the paper was pretreated with 1% sulfuric acid at 140 °C, cellobiose and cellotriose were fermented by eliminating β-glucosidase, and then cellulase was added to promote saccharification to ethanol (Ingram, Conway, Clark, Sewell, & Preston, 1987). Ingram also reported the development of a recombinant *Escherichia coli* that expressed alcohol dehydrogenase and pyruvate decarboxylase from *Zymomonas mobilis* (Brooks & Ingram, 1995). The *E. coli* converted 00 g/L of glucose to 45 g/L of ethanol. It was reported that a recombinant sake yeast, *Saccharomyces cerevisiae* GRI-117-UK was prepared from *Aspergillus oryzae* by cloning β-glucosidase- and endoglucanase-encoding genes. The

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recombinant yeast produced ethanol from cellobiose and cellulose materials (Kotaka, Bando, et al., 2008a). Glucoamylase-displaying yeast, *S. cerevisiae*, was also constructed and it produced ethanol from liquefied starch (Kotaka, Sahara, et al., 2008b). The recombinant pYBGA1yeast was produced by encoding both the *bgl*A gene of *Aspergillus kawachii* IFO4308 and an uracil-encoding gene *Ura* 3, in *S. cerevisiae* YPH499, and it expressed β-glucosidase in both the extracellular fluid and cell wall (Iwashita, Todoroki, Kimura, Shimoi, & Ito, 1998). Therefore, the pYBGA1 yeast directly fermented cellobiose with a 1, 4-β-glucopyranosidic linkage to ethanol. Previously, we reported a method for a two-step acid hydrolysis of cellulose materials such as tissue paper, cotton, and sawdust to give a mixture of cello-oligosaccharides containing glucose, cellobiose, and higher cello-oligosaccharides, a 40 g/L solution of which was fermented with pYBGA1 yeast to give ethanol as high as 70% conversion and 19 g/L concentration (Uryu et al., 2006).

Cello-oligosaccharides are one of the key intermediates in the production of ethanol because cello-oligosaccharides are readily produced from cellulose under mild enzymatic saccharification compared to glucose production and fermentation by pYBGA1 yeast. In this study, we describe the direct saccharification and fermentation of cello-oligosaccharides, cellobiose, cellotriose, cellotetraose, and cellopentaose, to ethanol by using pYBGA1 yeast. We found that the direct saccharification and fermentation of cello-oligosaccharides proceeded smoothly to give ethanol in relatively good conversions and concentrations, and the process was monitored by high performance liquid chromatography (HPLC). This method using cello-oligosaccharides is a simple procedure to produce fuel ethanol.

2. Experimental

2.1. Measurement

Cell-oligosaccharides in the medium were identified by aqueous phase HPLC (column; Tosoh TSK-gel Amide-80, 7.6 mm × 250 mm eluted with acetonitrile aqueous solution at a flow rate of 0.5 mL/min) with a Tosoh RI detector. The ethanol concentration was recorded by gas chromatography (model; Shimadzu GC-8A; column: SE-30, 3.2 mm × 3.0 m, Shimadzu) fitted with a flame ionization detector and operated at column and injector temperatures of 60 and 130 °C, respectively. Nitrogen carrier gas was used at the flow rate of 25 mL/min.

2.2. Material

Recombinant pYBGA1 yeast was provided by the National Research Institute of Brewing. Commercially available cello-oligosaccharides, yeast extract, and peptone were purchased from Seikagaku Biobusiness Co. Ltd., Merck Chemicals, Japan, and Kyokuto Pharmaceutical Industrial Co. Ltd., Japan, respectively.

Preincubation and increase of pYBGA1 yeast on agarose medium at pH 5.0 with cellobiose as a carbon source were carried out for 5 days at 28.3 °C. Before use, the number of pYBGA1 yeast cells was counted using a cell counter.

2.3. Ethanol fermentation of cello-oligosaccharides

A typical procedure for the fermentation of cello-oligosaccharides is as follows. Yeast peptone cellobiose (YPC) medium was prepared from 10 g/L of yeast extract, 20 g/L of peptone and 50 g/L of cellobiose in 1000 mL of deionized water at pH 5.0, and then the medium was autoclaved for 20 min at 121 °C before use. Cellobiose was fermented of performed in 30 mL of the YPC medium with 1 × 108 cells/mL of pYBGA1 yeast for 120 h at 30 °C. A small amount of the fermentation solution was sampled every 12 h after the start of fermentation to measure quantitatively the ethanol produced by gas chromatography (GC) and the cellobiose remaining was determined by HPLC. Ethanol conversion was calculated from the theoretical concentration of ethanol from cellobiose in the feed. The results are demonstrated in Fig. 4B.

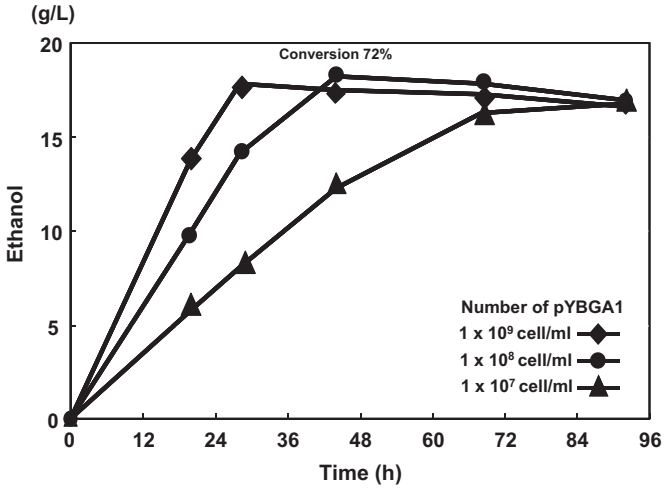

Fig. 1. Effect of number of pYBGA1 yeast cells on ethanol concentration at 28 °C in the fermentation of 50 g/L of glucose in the feed.

3. Results and discussion

3.1. Effect of pYBGA1 yeast concentration on ethanol fermentation

The recombinant pYBGA1 yeast expressed β-glucosidase in both the extracellular fluid and cell wall. Therefore, pYBGA1 yeast has ability to cause both saccharification of cello-oligosaccharides and ethanol fermentation. Before fermentation of cello-oligosaccharides, the suitable concentration of pYBGA1 yeast cells for fermentation of glucose was investigated, and the results are shown in Fig. 1. When 50 g/L of glucose in the feed was fermented with 1×10^9 cells/mL of pYBGA1 yeast at 28 °C, ethanol was produced at a 70% conversion rate and 17.8 g/L in 28 h. It was found that the highest conversion of glucose to ethanol was obtained with 1×10^8 cells/mL of pYBGA1 yeast to produce 72% conversion and 18 g/L after 44 h at 28 °C. Decreasing the number of yeast cells to 1×10^7 cells/mL decreased both the conversion of glucose and concentration of ethanol to 70% and 17 g/L, respectively, in 94 h. Accordingly, the concentration of pYBGA1 yeast cells 1×10^8 cells/mL, was selected for the direct saccharification of cello-oligosaccharides and fermentation to ethanol.

3.2. Effect of different temperatures

Based on the result of the optimal number of pYBGA1 yeast cells for the fermentation of glucose, direct saccharification and ethanol fermentation of cellobiose were performed. Fig. 2 shows the effect of the fermentation temperature on 50 g/L of cellobiose in the feed. The temperature varied from 26 °C to 33 °C. At 28 °C, cellobiose was converted to ethanol at a 76% conversion rate and 19 g/L of concentration after 96 h. A long fermentation time was necessary because pYBGA1 yeast first saccharified cellobiose to glucose and then the resulting glucose was fermented to ethanol. It was found that the conversion and concentration of ethanol increased with increasing temperature. At 30 °C, ethanol was produced at 80% conversion rate and 21 g/L after 72 h incubation. However, higher and lower