

Dilute acid hydrolysis and fermentation of corn fiber to xylitol

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Xylitol, a five-carbon sugar alcohol, has similar sweetening power as sucrose. It has unique pharmacological properties such as prevention of tooth decay and ear infection in children; it is used as a sugar substitute for diabetic patients and in parenteral application to trauma patients. Xylitol is increasingly being used in chewing gums, candy, soft drinks, ice cream and oral hygiene products. It occurs naturally in low levels in fruits and vegetables but it is impractical to extract xylitol from these sources. Xylitol can be produced from the catalytic hydrogenation of xylose or xylose-rich hemicellulose hydrolysates. The chemical production route requires extensive purification to meet food and pharmaceutical standards and therefore the product is very expensive.

Xylitol can also be produced by microbial reduction of xylose or xylose-rich hydrolysates. Some yeasts, filamentous fungi, and bacteria can convert xylose to xylitol at low productivities and relatively low concentrations. However, the microbial route to xylitol production is environmentally friendly and research in this area is growing. In this paper, we report the dilute acid hydrolysis and fermentation of corn fiber hydrolysates using *Candida tropicalis* as the biocatalyst. The goal of this research is to develop efficient methods of microbial production of xylitol from corn fiber and other xylan-rich biomass feedstocks.

The corn fiber used for these studies was obtained from Cargill (Cargill Inc, Minneapolis, MN). The wet corn fiber was air-dried at room temperature, Wiley milled to pass –20-mesh, and analyzed for summative composition. The milled corn fiber was hydrolyzed with various concentrations of sulfuric acid (0.25, 0.5, 0.75 and 1% v/v). The hydrolysis times were 15, 30, 45, 60, 75, and 90 min at 121 °C. The dried corn fiber was slurried with dilute acid (15% solid w/v) using the above concentrations of dilute sulfuric acid. The slurries were autoclaved at 121 °C for the above hydrolysis times. The samples were analyzed for monomeric sugar content after the hydrolysis. A set of the dilute acid treated samples were neutralized and further hydrolyzed with commercial enzymes such as Spezyme CP, and Econase CES. These hydrolysates were also analyzed for monomeric sugar composition.

One set of the dilute acid hydrolysates were neutralized with calcium hydroxide and another set was overlimed with calcium hydroxide. Both sets of samples were fermented with *C. tropicalis* at various pHs, initial cell mass, and yeast extract concentrations. All fermentations were carried out in 250 mL Erlenmeyer flasks on a shaker platform at 130 rpm and 35 °C for 120 h.

The release of sugars (glucose, xylose, and arabinose) during corn fiber hydrolysis with dilute acid increased with both increase in hydrolysis time and concentration of the acid. The highest amounts of sugar released (expressed as a percent of the theoretical maximum) were 72%, 73%, and 90% respectively for glucose, xylose and arabinose. These yields were obtained using acid concentration of 0.75% v/v, and 60 min. hydrolysis at 121 °C. Furfural and hydroxymethylfurfural were also produced

during the hydrolysis. The concentrations of furfural and hydroxymethyl furfural increased with increased acid concentration and hydrolysis time.

The commercial enzyme preparations were capable of releasing sugars from the dilute acid treated corn fiber, but the increase in the concentration of the sugars above those released during the acid treatment were not significant. Thus, all fermentations were conducted on the dilute acid hydrolyzates obtained at 0.75% acid, and 60 min. hydrolysis at 121 °C.

Investigations on the reduction of microbial growth and xylitol inhibition showed that simple neutralization of the hydrolysate with Ca(OH)_2 combined with activated carbon treatment was more effective than overliming the substrate with calcium hydroxide. Calcium hydroxide overliming appeared to inhibit cell growth and xylitol formation. Calcium hydroxide neutralization without activated carbon treatment was effective in removing toxic components from the dilute hydrolysate solutions (18% xylose) and supported microbial growth and xylitol production. However, as the xylose concentration in the hydrolysate was increased by vacuum evaporation to 36% and 60%, the simple neutralization was ineffective in the removal of xylitol inhibitors. Neutralization followed by activated carbon treatment was found to be the most effective way to reduce the inhibition of xylitol formation in concentrated hydrolysates. In spite of this treatment, the yields of xylitol from the hydrolysates were lower than those obtained from the fermentation of mixed model sugars.

Fermentation of mixed model sugars with similar compositions as those found in the hydrolysates showed statistically significant differences between the two substrates in the yield of various products. In comparison to model sugars, the growth pattern of the microorganism were similar, however, the cell growth rate, xylitol yields and productivities were lower in the hydrolysates. Whereas the cells in the hydrolysate experienced a lag phase of 10 h, those in the model sugar medium did not experience any lag period for similar initial cell mass concentration. Although the xylose uptake rates were similar in both media, the xylose conversion efficiency was lower for the hydrolysate solution. Xylitol yields were 0.34 g/g and 0.23 g/g respectively for the mixed model sugars and the hydrolysate.

Interestingly, although the xylitol yields from the hydrolysates were lower than expected, the xylitol inhibitors did not affect ethanol production. The ethanol yield from the glucose component of the hydrolysate was 85% of the theoretical yield and in some cases it was over 100% because it appears this microorganism also converted some of the xylose to ethanol. Xylitol yields from the mixed model sugars were relatively low compared to xylitol production from xylose fermentation. We have shown in our previous studies that ethanol inhibits xylitol production. Thus, inhibition of the xylitol formation could be a combination of ethanol effect as well as toxic components in the hydrolysate. In order to improve xylitol yield from corn fiber hydrolysate, both the ethanol effect and other unknown xylitol inhibitors must be removed from the media.