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On Fermentability of Nafion Catalyzed Hemicellulose Hydrolyzates

Scientific Note

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INTRODUCTION

Production of alcohol and other chemicals from replenishable resources is currently attracting renewed attention. Many problems exist in the extraction of the sugars and in the subsequent bioconversion of the sugars to ethanol. Extraction of sugars from hemicellulose and cellulose requires a hydrolytic catalyst, acid or enzyme. In the case of acid catalyst, an inherent problem exists in that the hydrolysis reaction is accompanied by degradation of sugar product (*1,2*). Recently, there has been considerable interest in the use of superacids (better proton donors than pure H_2SO_4) for hydrolysis of polysaccharides. Hardt and Lamport (*3*) studied the saccharification of wood by hydrogen fluoride and found it to have a much higher catalytic activity than sulfuric acid. Superacid catalysts also exhibit higher selectivity toward the hydrolytic reaction. This study was undertaken to ascertain the fermentability of the hydrolyzates produced by a solid superacid, Nafion.

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METHODS

Preparation of Hydrolyzates

Hardwood particles of southern red oak were screened, and the 40–100 mesh fraction was used as feed material for hemicellulose hydrolysis. Southern red oak contains 20–30% hemicellulose (4).

The straight acid hydrolyzates were prepared in two stages. In the first stage, hardwood particles were mixed with 0.2% H_2SO_4 to give solid–liquid ratio of 1:4, and reacted in an autoclave for 40 min at 160°C. The liquid portion (hydrolyzate of hemicellulose containing oligomers) was separated from the reacted solid residue by filtration using Whatman paper No. 2. The hydrolyzate was further hydrolyzed at 120°C for 1 h to convert the oligomer content into monomers.

The Nafion (copolymer of tetrafluoroethylene and perfluoro 3,6-dioxa-4-methyl-7-octenesulfonyl fluoride; m.p.: 200°C; insoluble in water) hydrolyzates were also prepared in two stages. The prehydrolysis was carried out by simple autoclave steam treatment without the use of catalyst (pure water hydrolysis or an autohydrolysis). The process was carried out at 160°C for 50 min under solid-to-liquid ratio of 1:4. The liquid portion was further treated with solid superacid, Nafion, 40–60 mesh (Solution Technology, Mendenhall, PA) to convert the oligomer content in the hydrolyzate into monomers. This reaction was carried out at 100°C for 1 h using 25 g of Nafion/300 mL of hydrolyzates. The Nafion catalyst was regenerated by heating at 70°C for 1 h with 15–20% HNO_3. The regenerated catalyst was washed repeatedly with deionized water and reused.

Microorganism

A stock culture of *Pachysolen tannophilus* (NRRL Y-2460) was maintained on agar slant. The slant medium contained 3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g xylose, and 20 g agar/Liter. The inocula were prepared by transferring a loopful of microorganism from the slants to 1-L Erlenmeyer flasks containing 500 mL of growth media with 40 g/L of xylose.

Substrate

The pH of both hydrolyzates was raised to between 11–12 by adding lime for precipitation of inhibitory compounds. The hydrolyzate was further supplemented with nutrients to the final composition of 3 g yeast extract, 3 g malt extract, and 5 g peptone/L of hydrolyzate (5). Prior to filter-sterilization, the pH was brought down to 5.5.

Fermentation

Fermentation runs were carried out in 250-mL Erlenmeyer flasks equipped with Morton closures. The flasks containing 50 mL of the hy-

drolyzates were placed on a gyratory shaker bath. The fermentation conditions were kept at 32°C, initial pH 5.5., and 400 rpm agitation.

Analytical Methods

The cell concentration was determined by turbidity measurements. The turbidity was measured by a turbidimeter (Hach Chemical Co. Model 2100A). A dilution factor of 250 was used (0.1 mL of sample mixed with 25 mL of deionized water). Sugar concentrations were measured by HPLC (Waters Associates, Sugar Analyzer I). The LC column was packed with an ion-exchange resin (Bio-Rad Aminex Q-15S, Calcium form). Ethanol was measured by GC (Varian 3700), equipped with FID and Chromosorb 101 column.

RESULTS AND DISCUSSION

A series of experiments were conducted in order to compare the fermentability of acid and Nafion hydrolyzates having comparable initial sugar concentrations. Since hardwood hemicellulose is composed primarily of xylose (about 90%, the balance being arabinose, mannose, and glucose), only the xylose component was considered as product in data presentation. The autohydrolysis reaction condition chosen in this study (160°C, 50 min.) was obviously not at the optimum point. This reaction condition was in fact on the mild side as indicated by the low yield of xylose obtained after treatment with Nafion. Consequently, the xylose concentration of the Nafion hydrolyzate reached only 1.4% as opposed to 4% in acid hydrolyzate. However, it is important to note that the low yield of xylose is not to be taken as an indication of Nafion being a poor hydrolytic catalyst. On the contrary, Nafion was extremely efficient in hydrolyzing the oligomer content in the prehydrolyzates. The Nafion hydrolyzates were concentrated under partial vacuum to raise their xylose content to 4%.

Shown in Fig. 1 are cell growth data in each hydrolyzate. It is clearly seen that the cells in Nafion hydrolyzate exhibit significantly higher growth rate as well as higher maximum cell yield than those of acid hydrolyzate. Figure 2 depicts the xylose consumption and the ethanol production rate. In fermentation of H_2SO_4 hydrolyzate, although inhibitory compounds suppressed the growth, yeast cells at initial inoculum level were able to produce ethanol by fermentation. In the case of Nafion hydrolyzate fermentation, a complete xylose utilization occurred after 75 h. Also, yeast in Nafion hydrolyzate consumed ethanol after xylose was completely depleted. However, the yield of ethanol was lower because of the fact that more fraction of substrate was channeled into cell growth rather than ethanol production. This is not a problem in fermentation practice because cell growth can be easily suppressed by adjustment of fermentation con-

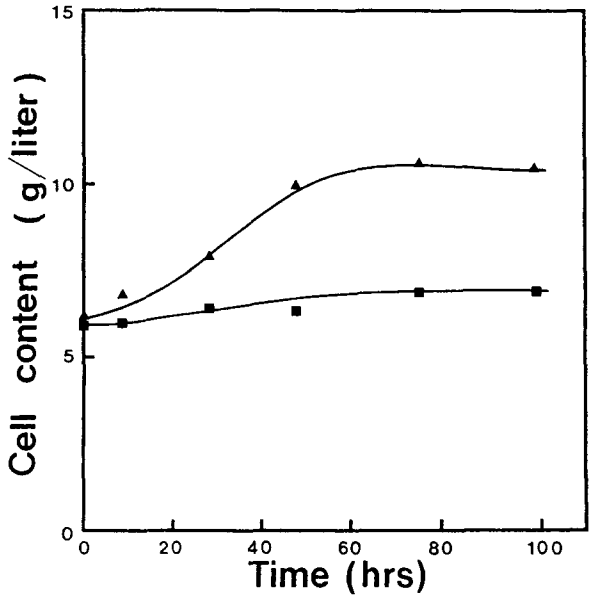

Fig. 1. Cell growth in acid and Nafion hydrolyzates. ■, Acid hydrolyzate; ▲, Nafion hydrolyzate.

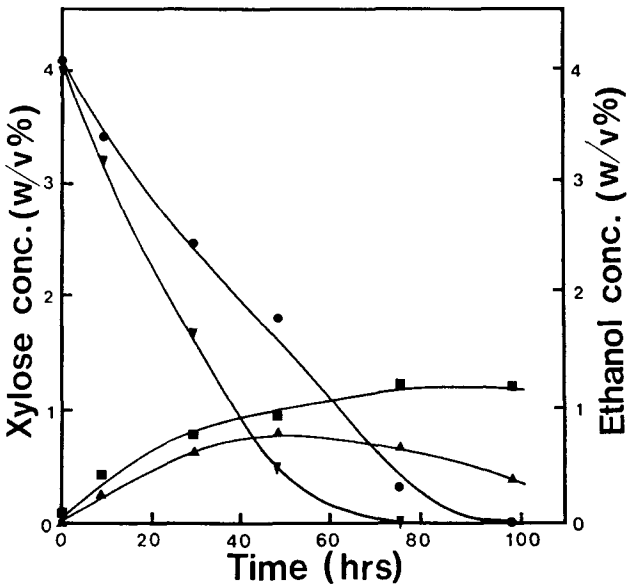

Fig. 2. Xylose consumption and ethanol production rates in acid and Nafion hydrolyzates. •, Acid hydrolyzate (xylose); ▼, Nafion hydrolyzate (xylose); ■, acid hydrolyzate (ethanol); ▲, Nafion hydrolyzate (ethanol).

ditions (aeration or nutrient limitation, for example). Since the survival and growth of the cells are primary criteria in evaluating the fermentability of acid/solid acid hydrolyzates, we conclude Nafion catalyzed hydrolysis may prove to be an effective means of converting hemicellulose into fermentable sugars.

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