RED OAK WOOD DERIVED INHIBITORS IN THE ETHANOL FERMENTATION OF XYLOSE BY *Pichia stipitis* CBS 5776

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SUMMARY

Xylose, the predominant sugar in red oak prehydrolysate, is fermented to ethanol by *Pichia stipitis* CBS 5776. Toxic model compounds derived from red oak hemicelluloses, lignin, and extractives inhibited the fermentation. Treatment of the prehydrolysate with molecular sieve and mixed bed ion resins facilitated the ethanol fermentation giving about 10 g/l ethanol from 32 g/l initial xylose. Fermentation inhibitors derived from red oak lignin and extractives were identified.

INTRODUCTION

The prehydrolysate generated from hardwoods by dissolving pulping processes consists mainly of xylose and limited fractions of lignin, extractives, and furfural (a degradation by-product of xylose; Rydholm, 1965). The prehydrolysate also contains acetic acid which is liberated from naturally occurring acetylated hemicelluloses. Thus, the potential inhibitors of the ethanol fermentation from xylose-rich red oak prehydrolysate are furfural, acetic acid, and compounds derived from lignin and extractives. The inhibitory effects of products generated in cellulose solvolysis on the subsequent fermentation have been studied (Pfeifer et al., 1984; Clark and Mackie, 1984). However, no work on the toxicity of compounds derived from the hemicellulose hydrolysis is available.

Recently, the yeast *Pichia stipitis*, under anaerobic conditions, was found to yield 0.40 gram ethanol per gram of xylose consumed (initial xylose: 30 g/l), which was higher than that obtained for *Pachysolen* and *Candida* sp. (Dellweg et al., 1984).

The objectives of the present investigation were thus: 1) to examine the fermentability of xylose by *Pichia stipitis* CBS 5776 under aerobic conditions, 2) to study the inhibitory effects of inhibitor model compounds derived from red oak hemicelluloses, lignin, and extractives, 3) to find suitable treatments for red oak prehydrolysate so that it can be fermented to ethanol, and 4) to identify the inhibitors from red oak lignin and extractives.

MATERIALS AND METHODS

Prehydrolysate of red oak wood. Commercially sized red oak (*Quercus falcata*) wood chips from Tennessee Valley Authority (moisture: about 30%) were cooked with distilled water at 170°C for one hour in a 2 gallon Parr reactor. Water to wood ratio was 3:5:1 (weight basis). After cooling and filtration, the filtrate was treated with 15 g/l sulfuric acid at 120°C for 45 minutes. The resulting liquor was the red oak prehydrolysate used throughout this work.
Microorganism and inoculum composition. The yeast Pichia stipitis CBS 5776 was obtained from Centraalbureau voor Schimmelcultures, Yeast Division, Delft, the Netherlands. Inoculum was composed of (for 1 liter) malt extract (3g), yeast extract (3g), peptone (5g), and xylose (10g), and sterilized at 120°C for 15 minutes. For the fermentations of xylose, inoculation volumes were 50 ml and incubated at 30°C for two days (0.4 g/l yeast). For the fermentations of red oak prehydrolysate, inoculation volumes were 800 ml and incubated for four days (1.1 g/l yeast). Inoculation was centrifuged and a part of xylose solution or prehydrolysate was used to transfer the yeast into fermentor.

Fermentor and fermentation conditions. All fermentations were carried out in a laboratory-made fermentor (Tran and Chambers, 1985) at 30°C with an aeration rate of 200 ml/min. pH was 5.0 for the xylose fermentations and 6.0 for the prehydrolysate fermentations. It was maintained with 5N sodium hydroxide or 0.02N sulfuric acid. Fermentation volume was 500 ml.

Inhibitory effects of model compounds. Compounds representing red oak hemicellulose degradation products were furfural and acetic acid (Fisher Scientific) which were redistilled. For red oak lignin derived products, model compounds were vanillin, syringaldehyde, vanillic acid, and syringic acid. Extractives model compounds were palmitic acid, caproic acid, caprylic acid, and pelargonic acid. Lignin and extractives model compounds were commercially purchased (Sigma Chemical) and used without further purification. Each of the above compounds was mixed with solutions containing 50 g/l xylose and nutrients (as control) prior to sterilization and fermentation.

Treatments of red oak prehydrolysate. Five treatments were carried out. Treatment A: prehydrolysate (600 ml) was stirred with molecular sieve (60 g) type S-115 (Union Carhide) at room temperature for one hour, filtered, adjusted with lime to pH 10.0, filtered, then adjusted again with sulfuric acid to pH 6.0. Treatment B; the similar procedure was applied for mixed bed ion resins AG-501-X8(0)(Bio-Rad). Treatment C: prehydrolysate was neutralized to pH 6.0 with lime and filtered. Recycled yeast (4.4 g/l) from the run of treatment B was then used as inoculation. Treatment D: the same as treatment A but without the step of stirring with molecular sieve. Treatment E: prehydrolysate was adjusted to pH 5.0 with lime, filtered, added with sodium sulfite (2%, based on xylose content), then adjusted again to pH 6.0 with lime. The treated prehydrolysates were used without further sterilization.

Preparation of petroleum ether and chloroform extracts. Petroleum ether extracts (0.08% yield) were prepared and derivatized as described elsewhere (Tran and Chambers, 1985). The chloroform extracts were prepared as follows. Prehydrolysate from extractive-free red oak wood (Tappi, 1959) (500 ml) was extracted with chloroform (120 ml × 3). The combined chloroform extracts were dried overnight over anhydrous sodium sulfate and evaporated to dryness in vacuo (50°C)(0.40% yield). The residue was then dissolved in dioxane (400μl) and silylated at room temperature overnight with pyridine (40μl) and bis(trimethylsilyl) trifluoroacetamide (200 μl). The derivatized petroleum ether and chloroform extracts were analyzed by gas chromatography/mass spectroscopy for the respective extractives and lignin derivatives.

Analytical. Ethanol, acetic acid, and furfural were analyzed on a Varian gas chromatograph model 3700 using a 1m glass column packed with Chromosorb 101 (60/80 mesh). Xylose was determined using a Waters Sugar