The nature of inhibitory materials present in pretreated lignocellulosic substrates which inhibit the enzymatic hydrolysis of cellulose

Mary Mes-Hartree and J.N. Saddler*

Biotechnology Group, Forintek Canada Corp., 800 Montreal Road
Ottawa, Ontario, Canada. K1G 3Z5

Summary

Wheat straw and aspen wood chips were pretreated by steam explosion and the various fractions were assayed for the presence of materials which inhibited the enzymatic hydrolysis of the cellulose component of the lignocellulosic substrates to glucose. The inhibitory material could be removed from all of the fractions by simple water extraction. The inhibitory substances were shown to primarily inhibit the β-glucosidase component of the cellulase complex of Trichoderma harzianum E58. The furan derivatives, furfural and hydroxymethyl furfural were not inhibitory at concentrations normally found in steam exploded lignocellulosic substrates.

Introduction

Before most native lignocellulosic substrates can be enzymatically hydrolysed to fermentable sugars there must first be direct physical contact between the cellulosic microfibrils and the enzyme complex. Therefore the substrates must first be pretreated to increase the accessibility of the substrate to the cellulase enzymes. One method which we and other workers (Jurasek, 1979; Taylor, 1980; Saddler et al, 1982) have been using is steam explosion. Steam explosion essentially includes both physical and chemical pretreatments which effectively catalyze the depolymerization of the hemicellulose and lignin. A variety of temperatures and retention times have been advocated by other workers using steam explosion (Su et al, 1980; Andren et al, 1975; Puls and Dietrichs, 1980).

Despite the wide range of conditions tested by these workers, most of them encountered problems of inhibitory materials being produced which restricted either the enzymatic hydrolyses of the pretreated substrate or the fermentability of the derived sugars (Chahal, et al 1982; Mes-Hartree et al, 1983). In work described in this paper we studied the effect that steam pretreatment had on the production of these inhibitors as well as trying to better define their nature.

Materials and Methods

Substrates. Wheat straw and aspen wood chips were steam exploded using a high-pressure gun with a 250 cm³ capacity (Saddler et al, 1982). The substrates were exposed to saturated steam at 560 psi (250°C) for 20 seconds. Some of the substrates were soaked in mild H₂SO₄, using vacuum impregnation, for 24h at 22°C, prior to steam explosion. Some of the pretreated residues were also further treated by extraction with 9 volumes of water for 2 hours at room temperature. Solka floc was purchased from Brown and Co. Berlin, N.H., U.S.A. Furfural and
Assays Total reducing sugars were estimated using the dinitrosalicylic acid assay (Miller, 1959). Glucose was determined colorimetrically by the glucostat enzyme assay (Raabo and Terkildsen, 1960). Individual sugars were assayed by high performance liquid chromatography (HPLC) equipped with a BioRad Aminex hpx-87P column which used water at a flow rate of 0.6 ml/min as the solvent. The column was maintained at 85°C and the refractive index detector maintained at a constant temperature.

Enzymatic hydrolysis procedure: The lignocellulosic substrates were incubated at a 5% substrate concentration (equivalent dry weight) with cellulase from Trichoderma harzianum E58 at a concentration of 30 IU filter paper activity per gram dry weight of residue. The cellulase profile and normal filter paper activity detected in the culture filtrates of T. harzianum E58 have been described previously (Saddler, 1982). Hydrolysis of the pretreated substrates and solka floc was performed at 45°C for 24 hours. The inhibition that various materials such as furfural might have on the enzymatic hydrolysis was assayed by supplementing the reaction with a specified amount of this material at the beginning of the hydrolysis. The water extract from steam exploded aspen was added to the reaction on a volume to volume ratio, i.e. 10% v/v was equivalent to 10 ml of the water washings added to 90 ml of the enzymatic reaction.

Results

Previously we had shown that when various lignocellulosic materials were pretreated by steam explosion, inhibitors were produced which restricted the enzymatic hydrolysis and fermentation of the cellulose and hemicellulose derived sugars to liquid fuels (Yu et al, 1982; Mes-Hartree et al, 1983; Saddler and Brownell, 1983). To try to further elucidate the nature of the materials which were inhibiting the enzymatic hydrolysis of lignocellulosic substrates, various steam exploded aspen wood and wheat straw fractions were assayed. Both steam exploded aspen wood and wheat straw were shown to contain substances which interfered with the efficient enzymatic hydrolysis of these substrates (Table 1). The percent conversion of the steam-exploded substrates to reducing sugars was comparable to what was obtained when solka floc was used as the substrate, when the lower cellulose content of the pretreated substrates was taken into consideration. The amount of glucose released from the pretreated substrates however was significantly lower. It was noted that the enzymatic hydrolysis of solka floc resulted in a reducing sugars to glucose ratio of 1.3 while this ratio was as high as 4.0 when some of the pretreated fractions were used as substrates. Previously we had found that soaking various lignocellulosic substrates in mild acid prior to steam explosion resulted in enhanced digestibility of the substrates. Although the conversion of the lignocellulosic substrate to reducing sugars was increased by prior acid soaking, the ratio of reducing sugars to glucose was significantly higher. When these fractions were water washed, the inhibitory material was removed and the ratio was similar to that found with the commercial cellulose. To verify that the inhibitory material was present in the water washings, various amounts of this material were added to a control hydrolysis using 5% solka floc as the substrate (Figure 1). It was found that the addition of as little as 2.5% of the water washings resulted in an increase in the reducing sugars to glucose ratio to 1.9.