Liquid Hot Water (LHW) Pretreatment of Alfalfa Fiber Destined for Ethanol Production

R.G. Koegel, H.K. Sreenath and R.J. Straub

Introduction

Alfalfa "fiber" referred to here is the result of wet fractionation of fresh alfalfa herbage into a juice fraction and a fiber fraction. While there are a number of other uses for this fiber fraction (Lu et al. 1979; Rowell et al. 1997), this study deals with its conversion to ethanol or organic acids by saccharification and fermentation.

It is frequently stated that the greatest single cost in producing ethanol from ligno-cellulosics is for the enzymes used to hydrolyze the fiber to fermentable sugars. It is therefore conventional wisdom that a chemical/thermal pretreatment to partially hydrolyze the fiber is necessary to improve processing economics. A number of such pretreatments have been proposed and studied. Many involve acids or bases used with elevated temperatures. Others involve softening with steam or liquid ammonia followed by an abrupt pressure drop to atmospheric. The pretreatment chosen for this study is referred to as liquid hot water, LHW, or "Aquasolv" (Mok and Antal 1992; VanWalsum et al. 1996). It consists of treating the feedstock with water at 220°C, pressurized to hold it in the liquid state, for two minutes. Advantages claimed for this process include: (1) no chemicals required which can ultimately lead to costs and waste products; (2) almost total hydrolysis of hemicellulose; and (3) partial hydrolysis of lignin. Enzymes are still needed for reducing the oligomers (short chains of sugars) in the pretreatment extract to monomers (single molecules) required by the fermentation organisms. Since hydrolysis of hemicellulose leads mainly to five-carbon sugars and hydrolysis of cellulose to six-carbon sugars, the fermentation organism(s) must be capable of fermenting both types of sugars. This dual capability is not frequently found but has been achieved in genetically engineered organisms.

Methods

The alfalfa "fiber" used in this research was the result of wet fractionation in which freshly cut alfalfa herbage was macerated using a rotary impact device and was immediately dejuiced by means of a screw press. The resulting fiber contained 70-75% of the dry matter of the original herbage. Its average composition (see Fig. 1) was cellulose, 33%; hemicellulose, 18%; lignin, 8%; protein, 11%; ash, 9%; and solubles, 22% (by difference). This material was air dried and stored for future use.

The pretreatment consisted of flowing preheated water at 220°C through 30 g of sample for two minutes. The water was held at sufficient pressure to insure that it remained in the liquid phase. Fig. 3 is a schematic of the apparatus. After the treatment chamber, the water with hydrolyzed dry matter was throttled through a valve to atmospheric pressure. This caused approximately one-third of the liquid to vaporize. After some initial treatments, a water-cooled condenser was added to allow retention of the vapor fraction for analysis. After the treatments, the fibrous residue was removed, oven-dried and stored for further analysis or treatment. The liquid "extract" was weighed and sampled to determine dry matter content. It was then stored under refrigeration for further use. Fiber and LHW products were saccharified using commercially available cellulases and pectinases. Fermentation was carried out using the genetically engineered yeast *Candida shehatae*, capable of fermenting both pentoses and hexose.

Results

The division of the fiber dry matter brought about by the LHW treatment is shown in Fig. 4. Almost 60% of the dry matter was extracted leaving around 40% of the original dry matter as fibrous residue. Only about 45% of the original dry matter was in the extract, however, leaving about 14% of the dry matter unaccounted for. It was initially assumed that this was the result of dry matter being volatilized. However, in later experiments to be reported elsewhere where 0.07% sulfuric acid was added to the treatment water

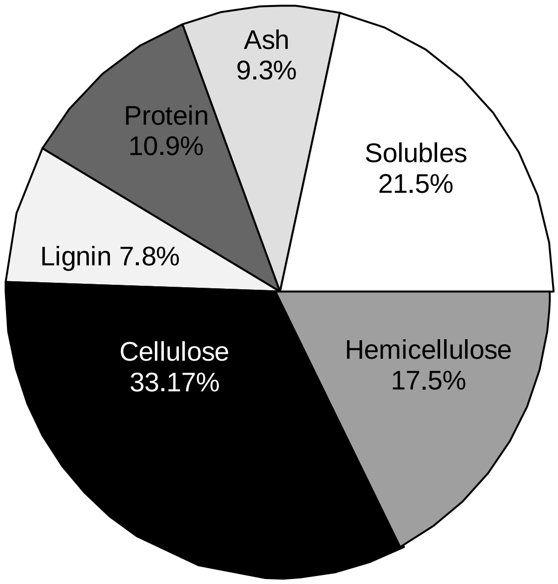

Figure 1. Representative composition of alfalfa "fiber" obtained by the wet fractionation process. N = 22 (circle area represents 100g)

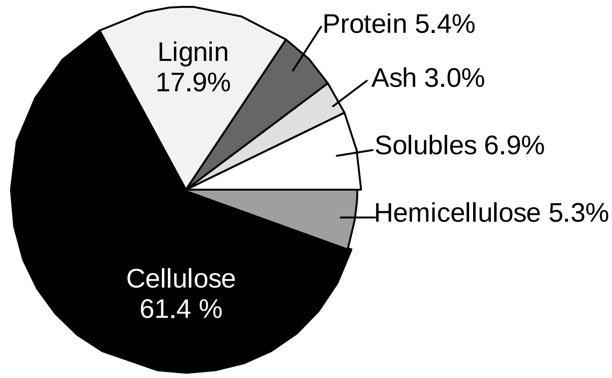

Figure 2. Representative composition of the fibrous residue resulting from the LHW treatment (circle area represents 41g, an average yield from 100g.

the sum of dry matters in the residue and the extract consistently exceeded the initial dry matter. This mass change anomaly cannot therefore be explained simply by volatilization. It could be the result of oxidation-reduction reactions, but in any case, requires further study.

The composition of the residue resulting from the LHW treatment is shown in Fig. 2. Since the circle areas in Figs. 1 and 2 are proportional to the masses of dry matter in the fiber and residue respectively, it is possible to see approximately to what degree various components were extracted. This degree of extraction of the three major fiber constituents—cellulose, hemicellulose, and lignin—is shown in Fig. 5. Hemicellulose is clearly the major constituent extracted. The results differ somewhat from those reported by Van Walsun et al. (1996) for bagasse and aspen. They reported extraction of 98-100% of the hemicellulose, 5-7% of the cellulose, and 37-65% of the lignin. The differences may be due to differences in the feedstock and/or slight processing parameter differences. For example, it is recognized that differences in cellulose crystallinity affect hydrolysis. It is also known that lignin may be solubilized and then reprecipitated under certain conditions.
The alfalfa fiber was enzymatically saccharified and fermented with and without the LHW pretreatment. In both cases,100 g of feedstock yielded 41-45 g of reducing sugars. In the case of no pretreatment, fermentation of these sugars gave an ethanol yield of about 14% rather than the "rule-of-thumb" 50%, indicating inhibition of the *Candida shehatae* used. In the case of sugars from LHW products, those resulting from the high-cellulose residue gave an ethanol yield of about 40% while the extract totally inhibited the organism, yielding no ethanol. Later work showed, however, that this extract could be fermentated to lactic acid using selected lactobacilli.

Conclusions

The LHW treatment was able to hydrolyze a large fraction of the hemicellulase (87%) and smaller fractions of the cellulose (24%) and lignin (6%). It did not appear, however, that this lead to more total fermentable sugars after enzymatic saccharification or to more ethanol. More study will be needed to determine whether the LHW treatment reduces the quantity of enzymes required. Inhibition of the fermentation organism is tentatively assumed to be caused primarily by acetic acid, a breakdown product of hemicellulose.

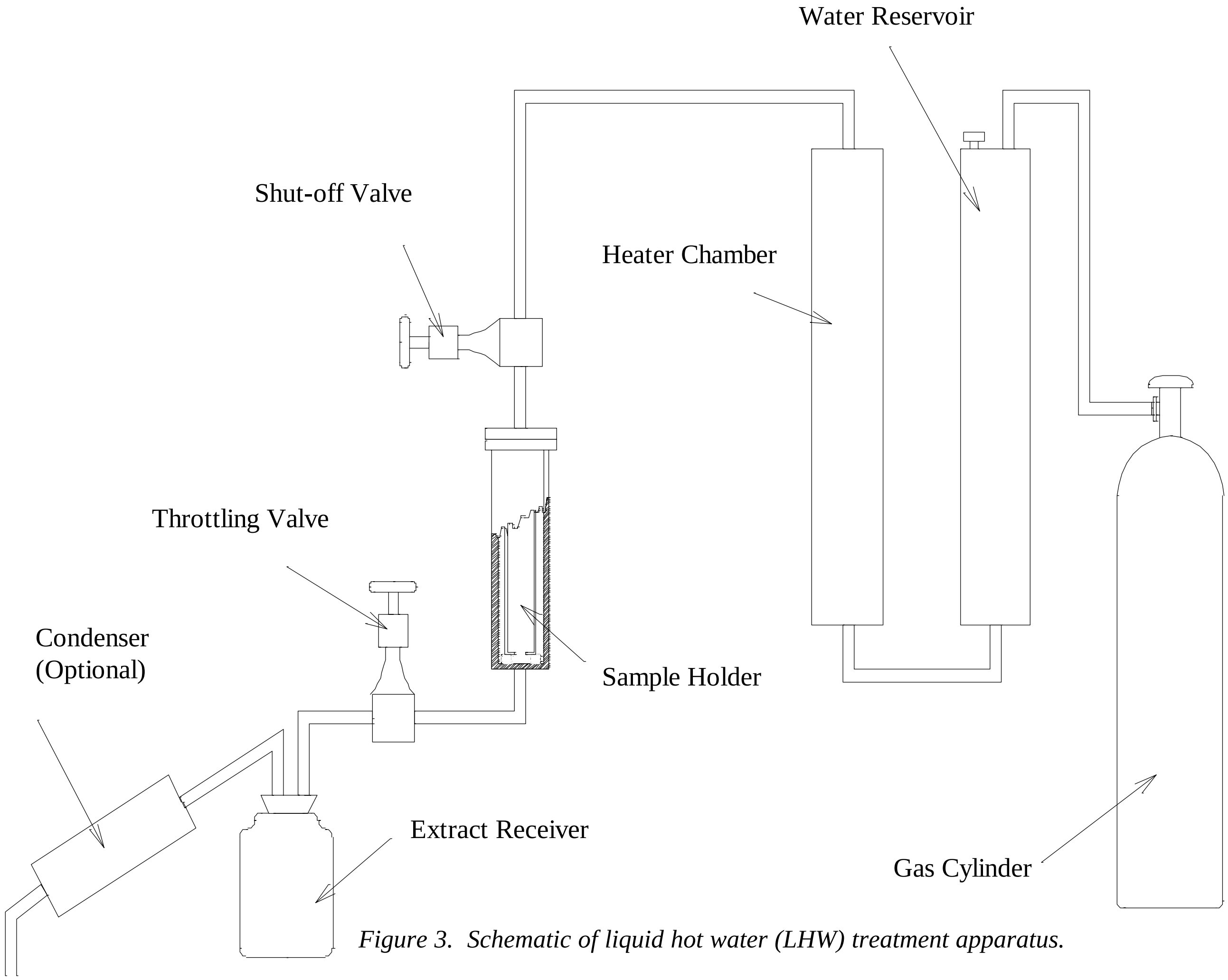

Figure 3. Schematic of liquid hot water (LHW) treatment apparatus.

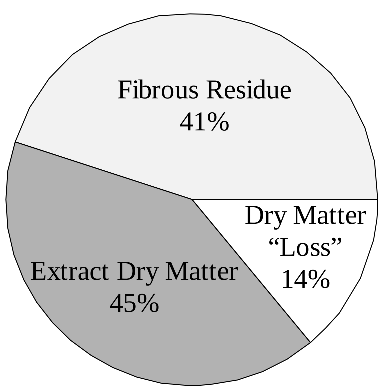

Figure 4. Division of dry matter in alfalfa fiber after LHW treatment. N = 22.

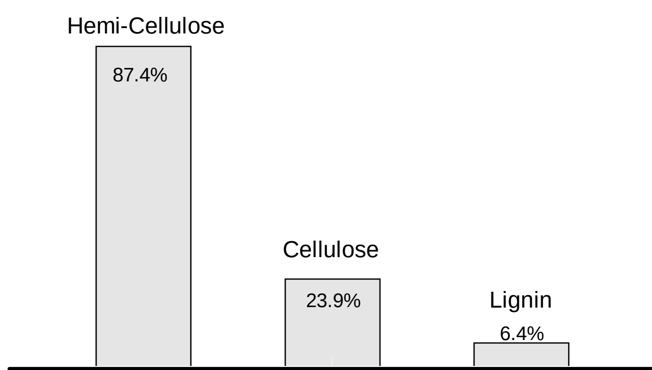

Figure 5. Percentage of the fiber constituents hydrolyzed in a LHW treatment.

References

Lu, C.D., Jorgenson, N.A. and Barrington, G.P. 1979. Wet fractionation process: preservation and utilization of pressed alfalfa forage. J. Dairy Sci. 62:1399-1407.

Mok, W.S. and Antal, M.J. 1992. Uncatalyzed solvolysis of whole biomass hemicellulose by hot compressed liquid water. Ind. Eng. Chem. Res. 31:1157-1161.

Rowell, R.M., Young, R.A. and Rowell, J.K. eds. 1997. Paper and composites from agro-based resources (book). Lewis Publishers.

Van Walsum, G.P., Allen, S.G., Spencer, M.J., Laser, M.S., Antal, M.J. and Lynd, L.R. 1996. Conversion of lignocellulosics pretreated with hot water to ethanol. Appl. Biochemistry and Biotechnology. 57/58:157-170.