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Consolidated Conversion of Hulled Barley into Fermentable Sugars Using Chemical, Thermal, and Enzymatic (CTE) Treatment

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Abstract A novel process using chemical, thermal, and enzymatic treatment for conversion of hulled barley into fermentable sugars was developed. The purpose of this process is to convert both lignocellulosic polysaccharides and starch in hulled barley grains into fermentable sugars simultaneously without a need for grinding and hull separation. In this study, hulled barley grains were treated with 0.1 and 1.0 wt.-% sulfuric acid at various temperatures ranging from 110 to 170 °C in a 63-ml flow-through packed-bed stainless steel reactor. After sulfuric acid pretreatment, simultaneous conversion of lignocellulose and starch in the barley grains into fermentable sugars was performed using an enzyme cocktail, which included α-amylase, glucoamylase, cellulase, and β-glucosidase. Both starch and non-starch polysaccharides in the pre-treated barley grains were readily converted to fermentable sugars. The treated hulled barley grains, including their hull, were completely hydrolyzed to fermentable sugars with recovery of almost 100% of the available glucose and xylose. The pretreatment conditions of this chemical, thermal, and enzymatic (CTE) process for achieving maximum yield of fermentable sugars were 1.0 wt.% sulfuric acid and 110 °C. In addition to starch, the acid pretreatment also retained most of the available proteins in solid form, which is essential for subsequent production of fuel ethanol and high protein distiller's dried grains with solubles co-product.

Keywords Hulled barley · Dilute sulfuric acid pretreatment · Fermentable sugar production · Enzyme hydrolysis · Lignocellulosic biomass conversion

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Nomenclature

Hulled barley	Barley kernels which retain their hulls during harvesting and storage
De-hulled barley	Hulled barley kernels that had their hulls removed by a physical method such as abrasion or another de-hulling technology
Hull-less barley	A type of barley containing a genetic hull-less trait. The barley hulls are loosely attached and are lost during the harvesting process

Introduction

The 2007 Energy Independence and Security Act sets a new Renewable Fuel Standard (RFS2) for the US fuel supply that will ultimately result in the use of about 36 billion gallons of renewable fuels per year by 2022 [1]. Currently, most fuel ethanol, the main renewable fuel available today, is made from Midwestern corn, the industry-preferred feedstock. Demand for ethanol in the East Coast is high and currently is met by railing in ethanol from the Midwest, which is expensive. The use of fuel for transportation also lowers the net energy value of the ethanol produced. Ethanol demand thus creates an immediate need for East Coast production plants, which can utilize regionally available feedstocks and also provide additional income to local growers. Because of the food versus fuel concern, ethanol from corn is capped at 15 billion gallons per year (GPY) in the RFS2. This restriction puts additional pressure on the industry to develop technically and economically convertible non-corn feedstocks whose use for ethanol production will not affect food supplies, production, or prices.

Whereas commercial production of second generation cellulosic ethanol is still several years away from economic viability, a feedstock alternate to corn that has the desired characteristics for production of ethanol using the current economically feasible technology is available. This feedstock is winter barley (also called "energy barley"), which can be considered as an advanced, "generation 1.5 feedstock." On the East Coast and in other regions with mild winters, winter barley can be planted immediately after corn harvest in the fall [2]. Winter barley germinates and grows slowly through the winter, but then grows quickly the next spring, and can be harvested in late May or early June, thus allowing a full-yield soybean crop to be grown and harvested the same year. This results in three cash crops for the producer in just 2 years with no conflicts with fuel versus food issues.

Since winter barley acts as a cover crop and prevents loss of nutrients and sediments into watersheds, its production will not only provide revenue and ethanol feedstock but will also improve the environment [3, 4]. In other regions in the USA, such as the upper Midwest and Northwest, spring barley is grown, primarily for malting purposes. Only the finest quality of barley is selected for malting purposes, leaving significant quantities of barley available for additional markets, such as fuel ethanol production. Until now, however, no attempts to make fuel ethanol from barley in the USA have been successful and sustainable. This is primarily because of three technical issues related to barley conversion:

1. Typical barley varieties have much lower (15–20% lower) levels of starch than corn, the "gold standard" fuel ethanol feedstock.
2. Barley has an abrasive hull that creates excessive wear on milling and processing equipment.
3. The presence of mixed-linkage β-glucans in barley results in a mash having extremely high viscosities, causing major difficulties in mixing during liquefaction and subsequent simultaneous saccharification and fermentation (SSF).

A current method to convert barley into fuel ethanol is to de-hull the barley to remove lignocellulosic fiber and then use enzymes to reduce the β-glucan-induced viscosity [5].

While these methods show promise, there is a need for an improved commercial process for conversion of barley into fuel ethanol, especially one that could result in conversion of both the starch and lignocellulosic sugars to fuel ethanol in one consolidated process.

The purpose of this study was to develop the aforementioned consolidated conversion of barley starch and non-starch polysaccharides into fermentable sugars for the production of fuel ethanol. With a combination of improved varieties of barley containing higher starch levels (~60–65%) and the simultaneous conversion of lignocellulosic polysaccharides also present in barley (~10%) into fermentable sugars, the yield of ethanol per bushel of barley could potentially match that of corn.

The consolidated conversion process developed in this study converted both lignocellulose and starch in hulled barley grains into fermentable sugars effectively using chemical and thermal pretreatment followed by enzymatic hydrolysis, which consists of two stages. The process thus is designated a chemical, thermal, and enzymatic (CTE) process. In the first stage, hulled barley is pretreated with dilute sulfuric acid at elevated temperatures. This pretreatment method was selected because the hydronium ion in the liquid phase can attack and hydrolyze β-glucans in the cell walls of the endosperm and the aleurone layer and also the hemicellulose in the hull [6–9]. Hydrolysis of the hemicellulose and partial removal of lignin would bring about a pretreatment effect on the lignocellulosic portion of the barley. Dilute acid pretreatment has been known as an effective method for hemicellulose hydrolysis and pretreatment of biomass [10–17]. Starch in grains must be heated to gelatinize (melt) starch before α-amylase and glucoamylase can effectively liquefy and saccharify it into glucose for conversion by the yeast *Saccharomyces cerevisiae* into ethanol. It is proposed that this thermal and chemical treatment also accomplishes the required gelatinization step so that starch, as well as lignocellulosic polysaccharides, are pretreated, liquefied, and become ready for enzymatic saccharification.

Materials and Methods

Barley

Nomini six-row winter barley was used for this study. The whole grains were used without any grinding or de-hulling. The cross-section of a typical barley grain is shown in Fig. 1, where the various components of the grain are identified. The kernel dry weight is typically made up of approximately the following: husk (hull) and pericarp, 7–14%; aleurone and associated testa, pigment strands, and some nuclear tissue, 14%; starchy endosperm, 55–70%; and embryo, 3% [18]. As shown in Fig. 1, the endosperm (starch) makes up most of the barley kernel. It is composed of starch granules protected by cell walls and a protein matrix. The endosperm is protected by the aleurone layer and hull. The composition of the selected hulled barley (Nomini) is shown in Table 1. The composition of the barley hull after the associated starch had been removed is shown in Table 2 [19]. The two major polysaccharides in the barley hull are glucan (33.6%) and xylan (30.5%). There is another minor component, arabinan (6.1%), which constitute side groups on the xylan molecule, which is more properly called arabinoxylan, the main component of barley hemicellulose.

Enzymes

The β-glucosidase enzyme, Novozyme 188 (Novo Inc., lot no. 11K1088), was purchased from Sigma-Aldrich (St. Louis, MO, USA). This enzyme had an activity of 750 cellobiose

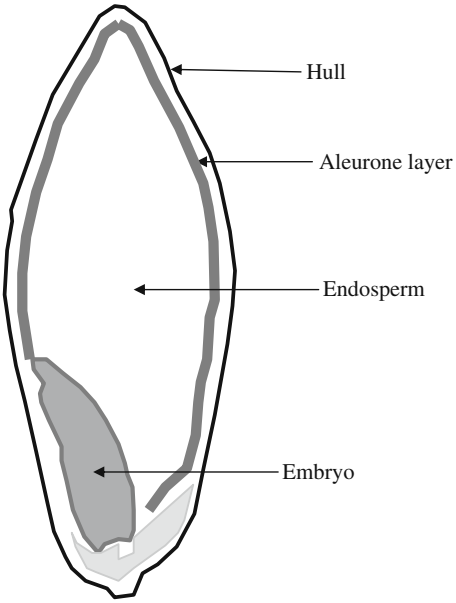

Fig. 1 Structure of barley kernel

units (CBU) per milliliter. All other enzymes were provided by Genencor, a Danisco Division. The cellulase enzyme, GC-220 (lot no. 301-04232-162) had an activity of 45 filter paper units (FPU) per milliliter. The α-amylase Spezyme Fred (no. 107-02285-003) had an activity of 18167 dextrinizing units (DU) per gram. The glucoamylase Optidex L-300 (lot no. 105-01232-001) had an activity of 289 glucoamylase units (GU) per gram.

Pretreatment Procedures

A schematic diagram of the pretreatment reactor and accessory components are shown in Fig. 2. The system consists of a burette, pump, heating oven [temperature programmable gas chromatography (GC) oven], flanged type stainless steel reactor with preheating coil, and sample cylinder, which also served as a backpressure vessel. The reactor was constructed out of 15.2 cm (6 in.) of SS-316 tubing with an I.D. of 2.3 cm (9/10 in.; 63 cm^3 of internal volume).

Table 1 Initial compositional analysis of hulled barley

Components	Hulled barley (g)
Starch	55.5
β-Glucan[a]	8.0
Xylan	3.7
Arabinan	0.7
Protein	9.9
Lignin[b]	3.0
Ash	2.8
Oil	2.8
Others	14.3

Data in the table are based on 100 g of the oven dry untreated whole barley grain, including endosperm, hull, and all the other components

[a] This contains all cellulose and any mixed linkage β-glucans

[b] Acid-insoluble lignin

Table 2 Initial compositional analysis of de-starched barley hull

Components	Barley hull (de-starched) (g)
β-Glucan[a]	33.6
Xylan	30.5
Galactan	0.6
Arabinan	6.1
Mannan	Trace
Lignin[b]	19.3
Ash	3.6
Starch	<0.8
Total	93.6

Data in the table are based on 100 g of the de-starched barley hull

[a] This contains all cellulose and any mixed linkage β-glucans

[b] Acid-insoluble lignin

In each experiment, 40 g air-dried hulled barley (~12% moisture) was packed in the reactor and sulfuric acid solution was pumped through the packed-bed flow-through reactor. Approximately 15 min of preheating was needed to reach the target temperature. Two concentrations of sulfuric acids, 0.1 and 1.0 wt.%, were used. Four different temperatures (110, 130, 150, and 170 °C) were tested at each acid concentration. The same pretreatment time (30 min) and flow rate (5 mL/min) were used in all experiments. This flow rate was chosen because previous studies (data not shown) with this reactor system showed that 5 mL/min was the minimum flow rate that did not produce detectable levels of sugar degradation products.

After completion of pretreatment, the solid samples were collected from the reactor and separated into two portions. One was dried in a moisture analyzer (Ohaus MB45, Pine Brook, NJ, USA) to determine weight loss and subsequently subjected to compositional analysis. The other was used for the enzymatic digestibility test. The liquid samples

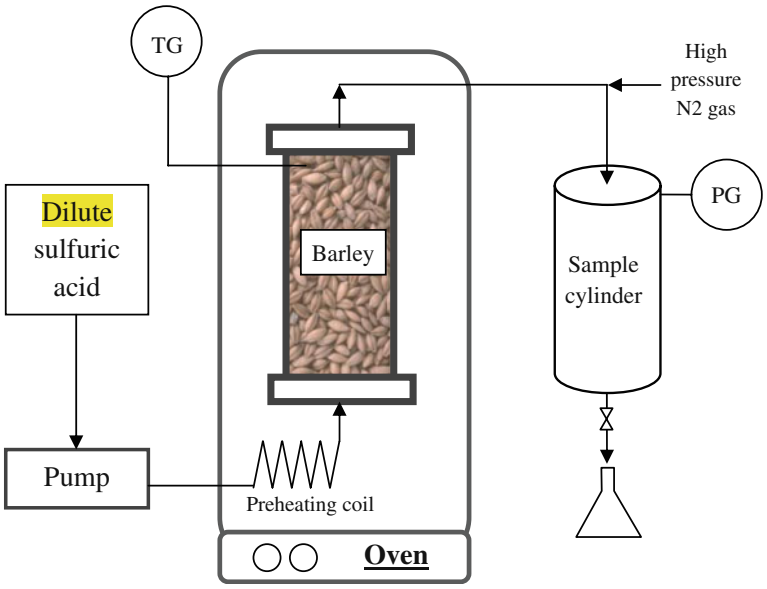

Fig. 2 Schematic of pretreatment reactor system. *TG* temperature gauge, *PG* pressure gauge

collected in the sample cylinder were subjected to liquid compositional analysis to determine the quantities of the solubilized carbohydrates.

Enzymatic Hydrolysis

Enzymatic hydrolysis of pretreated barley grains was conducted at 3% solid loading (3 g in 100 mL total volume) using 0.05 M citrate buffer at pH 5.0. Enzyme loadings were as follows: α-amylase, 0.1 mL (1,816 DU); glucoamylase, 0.1 mL (29 GAU); β-glucosidase, 0.1 mL (75 CBU); and cellulase, 0.25 mL (11 FPU). The cellulase loading was approximately 48 FPU/g cellulose plus xylan, which was higher than the typical loading used by other investigators (15–30 FPU/g cellulose). The high cellulase loading was used to ensure there would be excess enzyme. The reason was because in the mixture, in addition to the lignocellulosic components, i.e., cellulose plus xylan, there were other components such as starch and protein, especially starch at much higher concentrations, which might adsorb significant quantities of the cellulase and make it unavailable to its intended substrate. The enzyme hydrolysis of the pretreated barley was performed in duplicate. The materials used in these experiments were wet samples collected from the pretreatment experiments. The 250-mL screw-capped Erlenmeyer flasks containing the enzyme hydrolysis preparations were placed in an incubator shaker (Lab-line, 4,827F, Dubuque, IA, USA) maintained at 50 °C and shaken at 150 rpm. Samples were taken periodically (6, 12, 24, 48, 72, and 96 h) and analyzed for glucose, xylose, and cellobiose using high performance liquid chromatography (HPLC). Total released sugar after 72 h of hydrolysis was used to calculate the enzymatic hydrolysis yield.

Analytical Methods

To determine the composition of the Nomini barley grain, kernels were ground in a bench-scale Wiley mill, and the flour was used for analysis of starch, protein, β-glucan, oil, and ash. Starch was determined using an enzymatic procedure (total starch analysis procedure, Megazyme, Bray, Wicklow, Ireland) [20]. The same procedure also was used for starch analysis of the barley grains after pretreatment. Protein was determined in accordance with standard methods [21, 22]. The conversion factor used to obtain protein values for barley was 6.25 [23]. Beta-glucan was analyzed using a kit obtained from Megazyme International Ireland Ltd (Bray Business Park, County Wicklow, Ireland) according to ICC Standard Method 166 [24] and the instructions for the "streamlined method" provided by the manufacturer. This method conforms to standard methods [25, 26]. The oil content was estimated as described by Moreau et al. [27]. Four-gram samples were extracted with hexane in an accelerated solvent extractor (Dionex Corporation, Sunnyvale, CA, USA). The instrument was operated at 1,000 psi and a temperature of 100 °C for three 10-min cycles after which the hexane extract obtained was dried under a stream of nitrogen and oil content determined gravimetrically. The ash content was determined by heating barley flour in a muffle furnace at 550 °C for about 16–20 h until a light gray ash is obtained [28].

To determine the composition of the lignocellulosic components, the barley grains were first subjected to a mechanical de-hulling procedure. The weights of the two fractions, i.e., the hull and the de-hulled kernels, were determined. The hulls were then taken through a two-step enzymatic de-starching process to remove the residual starch in order to avoid interference in assays for cellulose and other glucans with glucose from cellulose [19]. The individual carbohydrate components and lignin content of the destarched hulls were

determined by the National Renewable Energy Laboratory (Golden, CO, USA) standard method [29]. The glucan and xylan results then were converted back to percentages of the whole barley grains as reported in Table 1 previously. The sugars in the liquid coming out of the pretreatment reactor were determined after secondary acid hydrolysis to account for the oligomer contents. The conditions of the secondary hydrolysis were 4 wt.% sulfuric acid and 121 °C for 1 h.

Each sample was run in duplicate. Sugars were determined by HPLC. The system was an ISCO model 2350 (Lincoln, NE, USA) using deionized water as solvent at 0.6 mL/min combined with an Aminex® HPX-87P column (Bio-Rad Laboratories, Hercules, CA, USA) operated at 85 °C and an HP 1047A refractive index (RI) detector (Hewlett Packard, Palo Alto, CA, USA). The software used for data analysis was Chrom Perfect® Spirit version 4 build 17 (Justice Laboratory Software, Auchtermuchty, Fife, UK).

Results and Discussion

The results of dilute sulfuric acid pretreatment of whole barley grains and subsequent enzyme hydrolysis of the pretreated materials are summarized in Table 3. These results are normalized to 100 g dry untreated barley grains.

Pretreatment of Barley Using Dilute Sulfuric Acid

As shown in Table 3, 100 g dry untreated barley grains contained 67 g fermentable sugars. In both cases where 1.0 and 0.1 wt.% sulfuric acid were used, as temperature was increased, the solid remaining after pretreatment (SR) decreased significantly. In fact, the relationships were almost linear. In the pretreatment using 0.1 wt.% sulfuric acid, the SR value was reduced from 97.3 g at 110 °C to 35.5 g at 170 °C. In the pretreatment using 1.0 wt.% sulfuric acid, these SR values were 92.1 and 27.4 g, respectively. These results also indicated higher loss of solids when the sulfuric acid concentration was increased from 0.1 to 1.0 wt.%. The decreases in SR when temperature and acid concentration were increased corresponded to the decreases in starch contents in the pretreated grains and the increases in glucose levels in the liquid coming out of the reactor. These observations clearly indicated that more severe conditions caused higher degrees of starch degradation. However, under the least severe conditions, very little losses of starch were observed. With the pretreatment using 0.1 wt.% sulfuric acid at 110 °C, 54.1 g starch, i.e., 97.8% of the initial quantity, was preserved. In the pretreatment using 1.0 wt.% sulfuric acid at 110 °C, 53.8 g of starch, i.e., 97.3% of the initial quantity, remained in the pretreated grains.

The untreated Nomini barley contains 9.9% protein and 2%–43% of the original protein was solubilized during pretreatment. Pretreatment at 110 °C, i.e., the lowest temperature used in this study, retained the highest amount of protein in the solid. At this temperature, almost 100% of proteins remained in the treated grains in both cases where 0.1 and 1.0 wt.% sulfuric acid were used. However, similar to starch, significant loss of proteins also was observed at higher pretreatment temperatures. With the pretreatment using 0.1 wt.% sulfuric acid, the protein content of the pretreated grains dropped from 9.7 g when the temperature was 110 °C to 5.6 g when the temperature was increased to 170 °C. A similar trend was observed in the pretreatment using 1.0 wt.% sulfuric acid. Whereas higher temperatures caused significant increases in loss of proteins, sulfuric acid concentrations did not show significant effect on protein loss. The protein contents of the grains pretreated at the same

Table 3 Mass balance table of acid treated hulled barley

Conditions	SR[a] (g)	Solid								Liquid[b]		Total CHO[c] (solid+liquid) (g)
		After pretreatment					Enzyme hydrolyzed sugars[d]			Monomer± oligomer		
		Starch (g)	β-Glucan[e] (g)	Xylan (g)	Protein (g)	Ash (g)	Glucan (g)	Xylan (g)	Total (g)	Glucan (g)	Xylan (g)	
Untreated	100	55.3	8.0	3.7	9.9	2.8	8.1	0.0	8.1	–	–	67.0
1.0 wt.% H_2SO_4												
170 °C	27.4	5.9	N/D[f]	0.1	5.6	1.0	12.8	~0.1	12.9	39.1	N/A[g]	N/A[g]
150 °C	46.2	19.6	N/D[f]	0.3	7.5	1.1	28.0	~0.3	28.4	27.8	N/A[g]	N/A[g]
130 °C	78.3	43.1	N/D[f]	0.7	8.9	1.7	55.4	~0.7	56.1	8.4	3.1	67.5
110 °C	92.1	53.8	N/D[f]	0.9	9.7	2.0	64.0	~0.9	64.9	0.5	2.2	67.6
0.1 wt.% H_2SO_4												
170 °C	35.5	12.0	N/D[f]	0.2	6.0	0.9	19.1	~0.2	19.3	34.5	N/A[g]	N/A[g]
150 °C	53.9	21.7	N/D[f]	0.4	8.1	1.1	30.3	~0.4	30.7	25.4	N/A[g]	N/A[g]
130 °C	83.3	43.7	N/D[f]	0.6	9.4	1.7	53.8	~0.5	54.3	6.5	1.2	62.0
110 °C	97.3	54.1	N/D[f]	0.6	9.6	2.0	60.1	~0.6	60.7	0.5	0.2	61.4

Data in the table are based on 100 g of the oven dry *untreated* whole barley grain, including endosperm, hull, and all the other components. Whole hulled barley, including ~12 wt% hull, was pretreated with sulfuric acid; the pretreatment conditions are described in "Materials and Methods"

[a] SR: solid remaining after pretreatment; for the untreated barley grains this is equal to 100 g

[b] Liquid coming out of the pretreatment reactor was hydrolyzed in 4% sulfuric acid at 121 °C for 1 h as described in "Materials and Methods"

[c] CHO: total carbohydrates. The values in this column are the sums of sugars in the hydrolysate after enzyme hydrolysis and sugars from liquid collected from the pretreatment reactor after secondary hydrolysis

[d] These are the quantities of the monomeric sugars generated in the enzyme hydrolysis of the pretreated barley grains. Details of the enzyme hydrolysis and conditions are described in the Materials and Methods section. The measured quantities of the monomeric sugars are converted back to their corresponding polymeric sources and normalized to 100 g dry starting barley grains, i.e. before pretreatment

[e] This contains all cellulose and any mixed linkage β-glucans

[f] Cellulose contents of the pretreated barley grains were not determined because it was decided that it would not be possible to obtain accurate results with small quantities of samples

[g] Data are not available

temperatures using different sulfuric acid concentrations, i.e., 0.1 and 1.0 wt.%, were very close to each other. The high preservation of both starch and proteins under the least severe pretreatment conditions is highly desirable for subsequent use of the pretreated grains in production of ethanol and a nutritional DDGS co-product.

Treatment at 110 °C retained most of the starch and protein in the solid (undissolved) portion, which is critical for subsequent production of ethanol and high protein DDGS (distiller's dried grains with solubles) co-product. After fermentation of sugar components, the DDGS co-product would have higher protein and lower fiber, ideal for higher value poultry and swine markets. In addition, difficult grinding of whole dry barley and high suspended solids that interfere with fermentation are removed, creating multiple opportunities for unique process configurations. Because barley ethanol plants are just now being planned in the USA, our new process could be incorporated into the initial design, obviating the need to retrofit conventional barley plants.

Enzyme Hydrolysis of Pretreated Barley

Glucose concentration profiles in enzyme hydrolysis of barley grains after pretreatment in 1.0 and 0.1 wt.% sulfuric acid at different temperatures are shown in Fig. 3. At each acid concentration, the initial rates of hydrolysis during the first 12 h were similar for all pretreatment temperatures. However, different final glucose yields were observed when the pretreatment temperature was varied as shown in Table 3. At higher temperatures, the yields of glucose were reduced. It is probable that higher temperatures in thermal and chemical pretreatments hydrolyzed and then decomposed more sugars in the barley grains, which resulted in lower carbohydrate contents after the pretreatment [30]. The data in Table 3 show that dilute sulfuric acid pretreatment followed by enzyme hydrolysis was capable of converting a very high percentage of all polysaccharides into fermentable sugars under

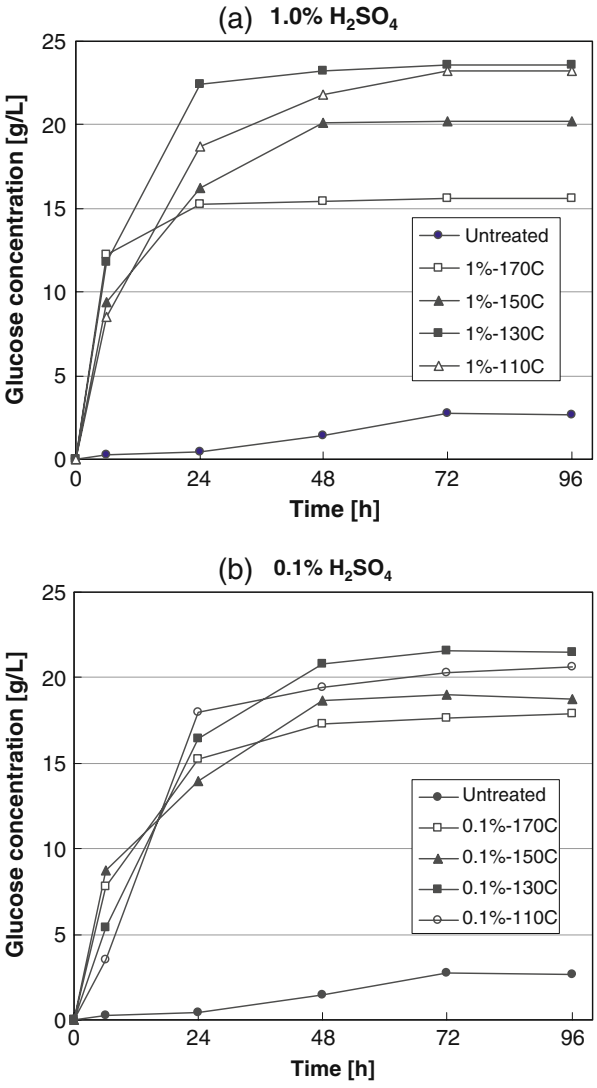

Fig. 3 Glucose concentration profile in enzyme hydrolysis. Whole hulled barley, including 12 wt.% hull, is pretreated with sulfuric acid and then enzymatically saccharified as described in "Materials and Methods"

selected pretreatment conditions. When 1.0 wt.% sulfuric acid and 110 °C were used for pretreatment, 64.9 g total fermentable sugars, i.e., 96.9% of the initial amount present in the barley grains, was recovered in the enzyme hydrolysis. When 0.1 wt.% sulfuric acid was used for pretreatment at the same temperature, 60.7 g total fermentable sugars, i.e., 90.6% of the initial amount, was recovered. It should be noted that in Table 3, the yields of sugars obtained in the enzyme hydrolysis are expressed as glucan and xylan and normalized to 100 g dry untreated barley grains. The conversion factor for glucose to glucan is 0.9, i.e., the measured quantity of glucose is multiplied by 0.9 to obtain the equivalent quantity of glucan. In the case of xylose to xylan, the conversion factor is 0.88. Enzyme hydrolysis of untreated barley grains was also carried out under the same conditions. The results in Fig. 3 and Table 3 clearly demonstrated considerable improvement of fermentable sugars production by the pretreatment process. Low yields of glucose from the untreated barley grains was expected because even in starch conversion, gelatinization of the starch granules, i.e., cooking at elevated temperatures (90–120 °C), is needed to facilitate hydrolysis by α-amylase and glucoamylase. Thus, the dilute sulfuric acid pretreatment served another purpose, i.e., starch gelatinization.

Comparison of Pretreatment Effects and Enzymatic Hydrolysis on Physical Properties of the Kernels

At all sulfuric acid concentrations, darkening of the sample was found to be more obvious at higher temperatures. Significant separation of the hulls was observed even at lower temperatures. After sulfuric acid treatment, hulls became somewhat gelatinous and were easily removed from kernels. Under optimal pretreatment conditions, for instance, 0.1% H_2SO_4 and 130 °C pretreatment, followed by enzymatic hydrolysis, the final "wort" contains mostly soluble sugars in the presence of small amounts of insoluble solids.

Practical Considerations

In the pretreatment process discussed in this report, both lignocellulosic polysaccharides and starch in hulled barley grains could be converted into fermentable sugars simultaneously without any hull separation and grinding steps. If these unit operations could also be omitted in commercial plants, significant savings in capital and operating costs could be realized. Conversion of the non-starch components to glucose would result in additional ethanol yield. If only starch in the grains is converted to ethanol, the theoretical maximum yield will be 397 L per dry metric ton (MT). If the non-starch polysaccharides excluding xylan and arabinan are also converted to ethanol, the yield will be 455 L per dry MT on a dry basis. This is equivalent to 14% increase in ethanol production, which is quite significant. In this analysis, xylan is excluded since it is assumed that the yeast *S. cerevisiae*, which cannot metabolize xylose, is used for ethanol fermentation. Conversion of β-glucans to glucose does not only result in additional ethanol production but also solves the problem of extremely high viscosity of the barley mash, which is caused by the solubilized β-glucans during the mashing operation. The removal of β-glucans through conversion to glucose also helps to avoid problems in downstream processing for ethanol recovery. In addition, the absence of β-glucans in the DDGS will make it suitable for use in the formulation of feed products for all animals, including both cattle and monogastric animals.

Production of ethanol from barley (endosperm+hull) can be significantly improved by hydrolyzing β-glucan in the earlier stage since the β-glucan would be problematic in terms

of high viscosity in the fermentor as noted above. Because the β-glucan was mainly present in the cell wall of endosperm and aleurone layer, the conditions for β-glucan removal could be optimized. Recently, a new barley ethanol process was developed, which uses both β-glucanases and β-glucosidase to reduce mash viscosity and convert all β-glucan into fermentable glucose [31]. This process results in higher ethanol yields due to fermentation of both starch and β-glucan. In this present process, both those species would be fermented to ethanol and also would be the cellulose present in the hull and in endosperm cell walls. The higher yields of ethanol and reduced capital costs for roller or hammer mills may more than offset the cost for the additional capital for pretreatment and additional enzymes.

Conclusion

Pretreatment of hulled barley using sulfuric acid at elevated temperature followed by enzyme hydrolysis was very effective in converting barley starch and non-starch polysaccharides into fermentable sugars. Pretreated hulled barley can be easily hydrolyzed by an enzyme cocktail without any grinding, de-hulling, or mechanical fractionation processes, and probably, additional pre- or post-fractionation of barley may increase the hydrolysis rate. Treatment conditions of CTE treatment for maximum fermentable sugars yield were 1.0 wt.% sulfuric acid, 110 °C, 30 min, 5 mL/min, and 4.3 mL 1 wt.% H_2SO_4 per gram of barley of liquid throughput.

The potential extra ethanol that a barley (grain) ethanol plant could produce by conversion of barley hull and endosperm cell walls to ethanol will be beneficial because of the extra revenue from additional ethanol. Furthermore, since more of the non-starch polysaccharides have been converted, the DDGS should have higher levels of protein than traditional barley DDGS, which would also give them higher value for animal feeds. Since with the proposed methods pretreated hulled barley can be converted to ethanol by SSF simultaneously and it can also contribute to the cost saving including elimination of de-hulling process, it can be an advantage for scale-up and potentially improve overall economics of bioconversion process of barley.

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References

1. The Library of Congress. Retrieved from http://thomas.loc.gov/cgi-bin/bdquery/z?d110:h.r.00006.
2. US Department of Agriculture National Agricultural Statistics Service (USDA-NASS) (2009). Crop acreage and yield US maps showing counties crop year 2008 United States. Retrieved May 2009 from http://www.nass.usda.gov/Charts_and_Maps/Crops_County/index.asp.
3. Chesapeake Bay Commission (2007). Biofuels and the Bay: getting it right to benefit farms, forests and the Chesapeake. Retrieved from http://www.chesbay.state.va.us/Publications/BiofuelsAndTheBay1.pdf.
4. Chesapeake Bay Commission (2008). Next-generation biofuels. Taking the policy lead from the nation. Retrieved from http://www.chesbay.state.va.us/Publications/nexgen%20biofuels1.pdf.
5. Gibreel, A., Sandercock, J. R., Lan, J., Goonewardene, L. A., Zijlstra, R. T., Curtis, J. M., et al. (2009). *Applied and Environmental Microbiology, 75*, 1363–1372.
6. Casebier, R. L., Hamilton, J. K., & Hegert, H. L. (1969). *Tappi, 52*(12), 2369–2377.
7. Fernandez-Bolanos, J., Felizon, B., Heredia, A., Guillen, R., & Jimenez, A. (1999). *Bioresource Technology, 68*, 121–132.

8. Lora, J. H., & Wayman, M. (1978). *Tappi, 61*, 47–50.
9. Burns, D. S., Ooshima, H., & Converse, A. O. (1989). *Applied Biochemistry and Biotechnology, 20/21*, 79–94.
10. Grohmann, K., Torget, R., & Himmel, M. (1985). *Biotechnology and Bioengineering Symposium, 15*, 59–80.
11. Kim, J. S., Lee, Y. Y., & Torget, R. W. (2001). *Applied Biochemistry and Biotechnology, 91–93*, 331–340.
12. Grethlein, H. E. (1985). *Bio/Technology, 3*, 155–160.
13. Allen, S. G., Schulman, D., Lichwa, J., Antal, M. J., Jennings, E., & Elander, R. (2001). *Industrial and Engineering Chemistry Research, 40*, 2352–2361.
14. Mok, W. S., Antal, M. J., Jr., & Varhegyi, G. (1992). *Industrial & Engineering Chemistry Research, 31* (1), 94–100.
15. Sun, Y., & Cheng, J. J. (2005). *Bioresource Technology, 96*(4), 1599–1606.
16. Nguyen, Q. A., Tucker, M. P., Keller, F. A., & Eddy, F. P. (2000). *Applied Biochemistry and Biotechnology, 84–86*, 561–576.
17. Saha, B., Iten, L. B., Cotta, M. A., & Wu, Y. V. (2005). *Process Biochemistry, 40*(12), 3693–3700.
18. Kulp, K., & Ponte, J. G., Jr. (Eds.) (2000). Handbook of cereal science and technology, 2nd edn. Boca Raton: CRC, p. 92.
19. Kim, T. H., Taylor, F., & Hicks, K. B. (2008). *Bioresource Technology, 99*, 5694–5702.
20. ICC Standard Method No. 168. Vienna: International Association for Cereal Science and Technology.
21. AOAC 990.03 (2000). Gaithersburg: Association of Official Agricultural Chemists.
22. AACC 46-30 (2003). St. Paul: American Association of Cereal Chemists.
23. AOAC 14.067 (2000). Gaithersburg: Association of Official Agricultural Chemists.
24. ICC Standard Method No. 166 (1998). International Association for Cereal Science and Technology 1998.
25. AOAC 995.16 (2000). Gaithersburg: Association of Official Agricultural Chemists.
26. AACC 32-33 (2003). St. Paul: American Association of Cereal Chemists 2003.
27. Moreau, R. A., Powell, M. J., & Singh, V. (2003). *Journal of the American Oil Chemists' Society, 80*, 1063–1067.
28. AACC 08-01 (2003). St. Paul: American Association of Cereal Chemists; 2003.
29. LAP-002, 003, 017, 019—Determination of structural carbohydrates and lignin in biomass (2004). Golden: National Renewable Energy Laboratory. Retrieved from http://www.nrel.gov/biomass/analytical_procedures.html.
30. Xiang, Q., Lee, Y. Y., & Torget, R. W. (2004). *Applied Biochemistry and Biotechnology, 113–116*, 1127–1138.
31. Nghiem, N., Hicks, K., Johnston, D., Senske, G., Kurantz, M., Li, M., et al. (2010). *Biotechnology for Biofuels*, 3:8. Retrieved from http://www.biotechnologyforbiofuels.com/content/3/1/8.