Appl Biochem Biotechnol (2011) 165:870–882
DOI 10.1007/s12010-011-9304-1

Scale-up of Ethanol Production from Winter Barley by the EDGE (Enhanced Dry Grind Enzymatic) Process in Fermentors up to 300 l

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Received: 9 February 2011 / Accepted: 1 June 2011 /
Published online: 11 June 2011

Abstract A fermentation process, which was designated the enhanced dry grind enzymatic (EDGE) process, has recently been developed for barley ethanol production. In the EDGE process, in addition to the enzymes normally required for starch hydrolysis, commercial β-glucanases were used to hydrolyze (1,3)(1,4)-β-D-glucans to smaller molecules, thus reducing the viscosity of the mash to levels sufficiently low to allow transport and mixing in commercial equipment. Another enzyme, a developmental β-glucosidase, then was used to hydrolyze the resulting oligomers to glucose, which subsequently was fermented to produce additional ethanol. The EDGE process was developed with Thoroughbred, a winter hulled barley, using a *shake flask* model. To move toward commercialization, it is necessary to prove that the developed process would be applicable to other barley varieties and also to demonstrate its scalability. Experiments were performed in 7.5, 70, and 300-l fermentors using Thoroughbred and Eve, a winter hull-less barley. It was shown that the process was scalable for both barley varieties. Low levels of glucose throughout the course of the fermentations demonstrated the high efficiency of the simultaneous saccharification and fermentation process. Final ethanol concentrations of 14% (*v*/*v*) were achieved for initial total solids of 28.5–30% (*w*/*w*), which gave an ethanol yield of 83–87% of the theoretical values. The distillers dried grains with solubles co-products contained very low levels of β-glucans and thus were suitable for use in feed formulations for all animal species.

Keywords Ethanol fermentation · Winter barley · β-Glucans · Process scale-up · Biorefinery

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Introduction

The inevitable depletion of fossil energy sources has stimulated worldwide interest in alternative and renewable fuels. Most recently, the need for a clean liquid fuel such as ethanol became more urgent after the environmentally disastrous oil spill in the Gulf of Mexico that was caused by explosion of an undersea oil well. In the USA, the 2007 Energy Independence and Security Act (EISA) mandated production of 36 billion gallons of renewable fuels by 2022, of which 22 billion gallons must be "Advanced Biofuels" made from non-corn feedstocks [1]. Ethanol production from corn in the USA has steadily increased during the last 15 years to reach 10.6 billion gallons in 2009 [2]. The increase in corn ethanol production is expected to continue. However, the 2007 EISA also limited the quantity of ethanol that can be produced from corn in the USA at 15 billion gallons per year to avoid negative impacts on feed and food markets. Thus, production of ethanol from renewable feedstocks other than corn is needed. Ethanol produced from lignocellulosic biomass can help meet the stated goal of the 2007 EISA, but the technology is not quite ready for commercialization. The main problem of cellulosic biomass ethanol production is the difficulty in feedstock conversion that results in uneconomical production cost. Starch conversion technology, on the other hand, has been successfully used for commercial production of ethanol for many years. Thus, a process for ethanol production that can use starch-based feedstocks other than corn is of great interest, especially when it is a winter grain, such as winter barley, that is grown on what would otherwise be fallow land and therefore does not compete with food and feed production during the summer growing season. For these reasons, ethanol from winter grains might qualify as an advanced biofuel, if a lifecycle analysis shows a 50% or greater reduction of greenhouse gas emissions compared to gasoline.

A process, which was designated the enhanced dry grind enzymatic (EDGE) process, has recently been developed in our laboratory for barley ethanol production [3]. One of the key issues of using barley for ethanol production is the high viscosity of the mash, which is caused by the (1,3)(1,4)-β-D-glucans in the grains when they are solubilized during the mashing. These β-glucans constitute the largest non-starch polysaccharide component of the endosperm cell wall and account for approximately 3.0% to 4.5% of the total grain weight [4]. In the EDGE process, in addition to the enzymes normally required for starch hydrolysis, commercial β-glucanases were used to hydrolyze (1,3)(1,4)-β-D-glucans to smaller molecules, thus reducing the viscosity of the mash to levels sufficiently low to allow transport and mixing in commercial equipment. Another enzyme, a developmental β-glucosidase, then was used to hydrolyze the resulting oligomers to glucose, which subsequently was fermented to produce additional ethanol [3]. The EDGE process was developed with Thoroughbred, a winter hulled barley variety, using a *shake flask* model. The optimum conditions determined in shake flask experiments were [3]:

(a) Liquefaction time of 2 h at 90 °C
(b) Enzymes required for liquefaction included SPEZYME® XTRA (thermostable α-amylase) and OPTIMASH™ BG (β-glucanase) at dosages of 0.30 and 0.13 kg/t dry solids, respectively
(c) Enzymes required for the simultaneous saccharification and fermentation (SSF) included FERMENZYME® L-400 (glucoamylase/protease mix) and a developmental β-glucosidase at dosages of 0.65 and 1.22 kg/t dry solids, respectively.

Under the optimum conditions, 30% of total dry solids could be used to produce 15% (*v*/*v*) ethanol, which gave a yield of 402 l/metric ton (dry basis) or 2.17 gal/53 lb bushel of

barley with 15% moisture [3]. The optimum conditions determined with Thoroughbred [3] also were applied to Eve, a hull-less barley variety. In shake flask experiments, similar ethanol yields (percent of theoretical value based on starch plus β-glucans) were obtained (unpublished results). After development of the EDGE process, our next objective was to test its scalability. It was of scientific as well as commercial interest to demonstrate the scalability of the process with both hulled and hull-less barley because of their different characteristics. Hull-less barley grains are very similar to their hulled counterpart, except the fact that their hulls come off readily during harvest. Thus, hull-less barley grains used in ethanol fermentation have higher starch content and lower fiber content compared to hulled barley grains (see Table 1 in the "Materials and Methods" section below). Higher starch content allows higher potential ethanol production at the same total solid levels. On the other hand, higher starch loading may require longer time for completion of the fermentation. We report here the results of experiments performed on the EDGE process scale-up using Thoroughbred and Eve in fermentors up to 300 l in size.

Materials and Methods

Materials

Thoroughbred (winter hulled barley) and Eve (winter hull-less barley), originally developed at Virginia Polytechnic Institute and State University, were grown by and obtained from the Virginia Foundation Seed Center, Mt. Holly, Virginia in 2005. Upon receipt, the grains were placed in a freezer for approximately 3 days to eliminate insects. The grains were subsequently stored in a low humidity room at ambient temperature (18–24 °C) and relative humidity below 25% until used. Four 50-lb bags from the same lot number were thoroughly mixed for 2 min using a tumbling dryer, operated without heat or vacuum. After mixing and sampling, the grains were divided equally into four plastic pails containing approximately 50 lb in each pail for storage. The composition of the two barley varieties was determined,

Table 1 Compositions of the two barley varieties Thoroughbred (hulled) and Eve (hull-less)

Component	Quantity (%)[b]	
	Thoroughbred (hulled barley)[c]	Eve (hull-less barley)
Moisture (whole kernels)	8.09±0.03	8.00±0.14
Oil[a]	1.92±0.06	1.87±0.09
Starch[a]	59.89±1.20	63.83±0.89
Protein[a]	7.60±0.03	10.06±0.12
β-Glucan[a]	3.90±0.05	4.08±0.08
Acid detergent fiber[a]	5.47±0.21	1.72±0.05
Neutral detergent fiber[a]	17.22±1.05	11.03±1.75
Crude fiber[a]	4.66±0.12	1.77±0.05
Ash[a]	2.32±0.0.02	1.74±0.02

[a] Dry basis

[b] Average of three determinations

[c] Data from Nghiem et al. [3]

and the results are summarized in Table 1. The methods used for the compositional analysis are described in the analytical section.

All of the enzymes, which included SPEZYME® XTRA (thermostable α-amylase, activity 14,000 U/g), OPTIMASH™ BG (β-glucanase, activity 10,300 U/g), OPTIMASH™ TBG (thermostable β-glucanase, activity 5,625 U/g), FERMENZYME® L-400 (glucoamylase/protease mix, activity 350 glucoamylase U/g), and a developmental β-glucosidase (activity 1,100 U/g), were provided by Genencor International (a Danisco division), Rochester, NY, USA. The enzymes were kept refrigerated at 4 °C.

Active Dry Ethanol Red was provided by Lesaffre Yeast Corporation, Milwaukee, WI, USA. The dry yeast powder was kept refrigerated at 4 °C. All chemicals were of reagent grades and purchased from Sigma-Aldrich, St. Louis, MO, USA.

Experimental Procedures

Fermentations in Bench-Scale Fermentors Both hulled (Thoroughbred) and hull-less (Eve) barley were studied in 7.5-l bench-scale fermentors. The barley grains were ground in a Wiley mill fitted with a 1-mm screen. The mash for ethanol fermentation was prepared in batches of 2,500 g total weight. The total solids content in the hulled barley mash was 30%, which was the same as the total solids contents normally used for making corn mash in commercial dry-grind ethanol fermentation. The total solid contents of the hull-less barley mash were set at 30%, which was the same as in the case of hulled barley, and also at 28.15% to give the same initial starch concentration as in the hulled barley mash. First, the moisture content of the ground barley was determined using the method described in the analytical section below. The quantities of ground barley needed to give the desired total solids contents, i.e., 700 g dry matter for the hull-less barley mash having 28.15% total dry solids and 750 g dry matter for the hulled and hull-less barley mashes having 30% total dry solids, then were added to a 4-l beaker containing de-ionized water needed to make a total weight of 2,500 g. The slurry was stirred with a mechanical agitator. The pH was adjusted to 5.2 with 2 M sulfuric acid, and the enzymes required for liquefaction were added. In the case of the hulled barley mash, these enzymes included SPEZYME® XTRA, which was added at 203 μl (0.50 g/kg starch), and OPTIMASH™ BG, which was added at 89 μl (3.33 g/kg β-glucans). In the case of the hull-less barley mash having 28.15% total solids, the same SPEZYME® XTRA dosage was used but the OPTIMASH™ BG dosage was reduced accordingly to match the slightly lower β-glucans level. In the case of the hull-less barley mash having 30% total solids, the dosages of both enzymes were increased accordingly to match the higher starch and β-glucans levels. Another enzyme, OPTIMASH™ TBG, was also added at 41 μl (1.47 g/kg β-glucans) since in preliminary tests it was observed that the viscosity of the mash was so high that the thermally stable β-glucanase OPTIMASH™ TBG was needed to sufficiently reduce it to allow stirring. The mash was heated on a hot plate and the heating rate was adjusted to maintain the desired temperature. The mash temperature was maintained at 90 °C for 2 h. During this time, small amounts of de-ionized water were intermittently added to compensate for evaporation loss. At the end of the starch liquefaction, the beaker was cooled in a water bath. When the temperature of the mash dropped to about 40 °C, the beaker was weighed and de-ionized water was added to bring the total weight back to 2,500 g. The mash was stirred and its pH adjusted to 3.8–4.0 with 2 M sulfuric acid. To the cooled hulled barley mash, two more enzyme products were added, which included FERMENZYME® L-400 at 443 μl (1.09 g/kg starch) and the developmental β-glucosidase at 1.60 ml (31.2 g/kg β-glucans), together with 1.0 g urea (0.4 g/kg total mash weight) to the hulled barley mash. The enzyme dosages

added to the hull-less barley mashes were adjusted accordingly to match their starch and β-glucans levels. Stirring of the mash was continued for 20 min to ensure complete dissolution of urea and uniform distribution of the enzyme. Then two batches of mash, i.e., 5,000 g total weight, were placed in a New Brunswick BioFlo 110 fermentor (Edison, NJ, USA), which had a total volume of 7.5 l. The active dry yeast was rehydrated by addition of 6.25 g to 125 ml de-ionized water and stirred for 30 min. The rehydrated yeast slurry then was added to the fermentor. The initial viable yeast count was about 5×10^6 per gram of mash. The SSF of the barley mash was carried out for 72 h. The temperature of the fermentor was maintained at 32 °C. Samples were taken at the end of the experiments for analysis of residual starch oligomeric components (maltotetraose, maltotriose, maltose), glucose, ethanol, and other fermentation co-products, which included glycerol, lactic acid, acetic acid, and succinic acid. The progress of the fermentation was followed by measuring the total carbon dioxide produced with a METRIS M250 gas meter (Actaris Metering Systems, Owenton, KY, USA), which used positive displacement of an internal liquid for gas volume measurements. The gas volumes were measured in liters. The meter was calibrated with air and displayed a sensitivity of one tenth of a liter. To avoid interferences with carbon dioxide production measurements, samples were not taken from the fermentors during the course of the fermentations. At the end of the experiment, the entire mash was removed from the fermentor, a small liquid sample (2 ml) was taken for high-performance liquid chromatography (HPLC) analysis of ethanol and other metabolites, and the mash was spread out on an aluminum tray and dried in an oven at 70 °C. The final distillers dried grains with solubles (DDGS) product was analyzed for starch, β-glucans, protein, phytic acid, and fiber contents. All of the experiments described in this section were performed in duplicates, and the average results were reported.

Fermentations in 70-l Fermentor An ABEC fermentor (Bethlehem, PA, USA) having a total volume of 70 l was used in these experiments. The total mash weight was 50 kg for both hulled and hull-less barley. The total solids contents (percent of total mash by weight) were 30% for the hulled barley and 28.15% for the hull-less barley. The enzyme dosages (in terms of grams per kilogram starch and grams per kilogram β-glucans) were the same as those described previously for the experiments performed in the 7.5-l fermentors. Instead of being prepared outside as in the previous case, the mash was prepared directly in the ABEC fermentor. The barley was ground in a Fitz mill equipped with a 2A screen having 2.36 mm openings. The ground barley and water were mixed in the fermentor and the pH was adjusted to 5.2 with concentrated sulfuric acid before SPEZYME® XTRA, and OPTIMASH™ BG were added. When the hull-less barley was used, OPTIMASH™ TBG also was added. The slurry was brought to 90 °C and maintained at that temperature for 2 h. Then the mash was cooled to 32 °C. The pH was adjusted to 3.9 with concentrated sulfuric acid before FERMENZYME® L-400 and β-glucosidase were added. Urea also was added to a final concentration of 0.4 g/kg total mash. The fermentor was inoculated with 250 ml of 5% rehydrated yeast, which was prepared as described previously. The temperature was maintained at 32 °C and the agitator speed at 200 rpm. The total fermentation time was 72 h, during which samples were taken at intervals for analysis of residual substrates and fermentation products as described previously. The progress of the fermentation was followed by measuring total CO_2 production as described previously. At the end of the fermentation, the CO_2 exhaust line was connected to a condenser and the mash was heated to boiling until about one half of the water had evaporated. Most of the ethanol produced also was evaporated during this heating period. The remaining mash then was dried in a rotary vacuum dryer using steam at about 80 °C for several days. This DDGS

co-product then was recovered for compositional analysis as described previously. Because of the lack of sufficient quantities of the barley grains, only one experiment was performed for each barley variety.

Fermentations in 300-l Fermentor After the 70-l fermentor, the fermentation process was scaled up further in a 300-l Biostat UD fermentor (B. Braun Biotech International, Bethlehem, PA, USA). Because of limited supply of hulled barley, only hull-less barley was used in these experiments. The experiments were performed in duplicates. The total mash weight was 250 kg and the total solids concentration was 28.15% of the total mash weight. The barley was ground in a Fitz mill equipped with a 2A screen having 2.36 mm openings. The mashing was performed directly in the fermentor. The mashing procedure and the enzyme dosages were the same as in the case of the 70-l fermentor. The inoculum was 1,250 ml of 5% rehydrated yeast. The fermentation time was 72 h during which samples were taken for analysis as described previously. The progress of the fermentation was followed by measuring total CO_2 production as described previously. At the end of the fermentation, the mash was boiled to a condenser until one half of the water had been evaporated. The remaining mash was dried in the rotary vacuum tumble dryer as described previously.

Analytical Methods

The analytical methods have been described previously [3], but for convenience of the readers, they are given in detail here. The moisture content of whole barley kernels was obtained by drying 10 g of barley at 130 °C for 20 h [5]. The moisture content of ground barley was determined by drying 2 g samples at 135 °C for 2 h [6].

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 [7]. The oil content of the ground barleys was estimated as described by Moreau et al. [8]. Barley was ground in a Wiley mill fitted with a 20 mesh screen, and 4-g 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 crude fat content of the DDGS was determined by weighing approximately 1 g of sample into an Ankom XT-4 Filter bag which was heat sealed. The bags were dried at 105 °C for 3 h and transferred to dessicator bags to cool. The dried bags (ten bags per run) were weighed and subsequently loaded into an Ankom XT-10 Fat Extractor (Ankom Technology, Macedon, NY, USA) and extracted with hexane (350 ml total) for 60 min at 90 °C. The bags were dried after extraction for at least 1 h at 105 °C, placed in dessicator bags to equilibrate to room temperature and weighed.

For starch analysis, barley samples were ground in a cyclone mill fitted with a 0.5 mm screen (Udy, Ft. Collins, CO, USA), and the flours were analyzed using a starch determination kit (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) [9]. The method was modified by use of a YSI 2700 Analyzer (YSI Incorporated, Yellow Springs, OH, USA) fitted with a YSI 2710 turntable for automated glucose determination of enzymatically hydrolyzed starch-containing samples.

The protein content of barley flour samples was determined in accordance with standard methods [10, 11]. The conversion factor used to obtain protein values for barley was 6.25 [12].

Barley β-glucan was analyzed using a kit obtained from Megazyme International Ireland Ltd. (Bray Business Park, Bray, Co. Wicklow, Ireland) according to ICC Standard Method 166 [13] and instructions for the "streamlined method" provided by the manufacturer. This method conforms to standard methods [14, 15].

Acid detergent fiber, neutral detergent fiber (NDF), and crude fiber were determined with an Ankom 2000 fiber analyzer (Ankom Technology, Macedon, NY, USA) as per the methods supplied by the manufacturer. Non-fiber carbohydrate (NFC) is defined as 100% dry matter minus % crude protein (CP) minus% NDF corrected for insoluble crude protein (NDICP) minus % fat and minus % ash [16]. Thus,

$$\mathrm{NFC} = 100\% - \%\mathrm{CP} - (\%\mathrm{NDF} - \%\mathrm{NDICP}) - \%\ \mathrm{Ash} - \%\ \mathrm{Fat}.$$

To determine the NDICP, the following method was used. Approximately 0.5 g of DDGS was weighed into an Ankom filter bag which was extracted with neutral detergent fiber reagent as instructed by the manufacturer of the Ankom Fiber Analyzer. After extraction and drying of the filter bags, the contents of the filter bags were weighed to determine % NDF and then were re-assayed for protein using the same method as used before for crude protein (copper catalyst/combustion method) as outlined in AOAC 990.03 [10] and AACC 46-30 [11] to determine the percentage of insoluble crude protein in the NDF fraction or NDICP. The value for % NDICP was then used to calculate the NFC value of the DDGS as shown above. Phytic acid content of the DDGS samples was determined by high-performance anion exchange chromatography at Genencor Analytical Laboratories, Palo Alto, CA, USA.

To determine the concentrations of fermentation products, samples taken from the fermentation flasks and fermentors were centrifuged and the supernatants were filtered through 0.2-μm filters. Ethanol concentrations were then determined by HPLC. The system was an ISCO model 2350 using 0.5% sulfuric acid as solvent at 0.6 ml/min combined with an Aminex® HPX-87H ion exclusion column (Bio-Rad Laboratories, Hercules, CA, USA) operated at 60 °C and an HP 1047A refractive index 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 the fermentation experiments are summarized in Table 2, which also include the calculated ethanol yields. The procedure used for calculation of the ethanol yield values has been described previously [3]. For the hulled barley in the 7.5-l fermentor, the ethanol yield was 84.2% of the theoretical value, which is slightly lower than the yield obtained previously in shake flask experiments under the same conditions (89.4%) [3]. Ethanol yield for the hull-less barley in the 7.5-l fermentor where the 28.15% solids mash was used was slightly higher than that obtained for the hulled barley (87.6% vs. 84.2%). The 28.15% solids hull-less barley mash contained the same amounts of starch and practically the same amounts of β-glucans as in the hulled barley mash but probably the lower total solids allowed the enzymes to work more effectively on hydrolysis of starch and β-glucans to produce more fermentable sugars for ethanol production. In the case of the 30% solids hull-less barley mash, all the enzyme dosages were increased proportionately to compensate for the higher starch and β-glucans levels in the mash. The ethanol yield in this case was statistically the same as in the case of the hulled barley mash as shown in Table 2. The major co-product in ethanol fermentation by *Saccharomyces cerevisiae* is carbon dioxide,

Table 2 Summary of fermentation results in 7.5-, 70-, and 300-l fermentors

	Fermentor size		
	7.5 l	70 l	300 l
Final ethanol (%, *v*/*v*)			
Thoroughbred	14.19±0.25	14.5	12.86±0.57
Eve—28.15% solids	14.42±0.15	14.04	
Eve—30% solids	14.64±0.23		
Yield (% theoretical)			
Thoroughbred	84.2±1.5	86.1	78.1±3.5
Eve—28.15% solids	87.6±0.9	83.4	
Eve—30% solids	82.4±1.3		
CO_2 production (l)			
Thoroughbred	244±1	2,519	12,483±491
Eve—28.15% solids	240±1	N/A	
Eve—30% solids	257±8		
Ethanol/CO_2 (mol/mol)			
Thoroughbred	0.97±0.02	1.0	0.87±0.08
Eve—28.15% solids	1.0±0.01	N/A	
Eve—30% solids	0.95±0.01		

which can be accurately measured by a gas meter. Although in *S. cerevisiae* in addition to ethanol synthesis the formation of succinic acid and acetic acid also involves carbon dioxide production, these two organic acids are produced in very small quantities. In our experiments, we routinely observed only about 2.5 g/l succinic acid and less than 0.5 g/l acetic acid. The calculated ethanol/CO_2 molar ratios shown in Table 2 indeed are equal to one, which is the expected value for the stoichiometric conversion of glucose to ethanol and carbon dioxide. Thus, measurement of carbon dioxide production can be used to follow the progress of ethanol fermentation. Figure 1 shows a typical CO_2 production vs. time curve in the case of hulled barley in the 7.5-l fermentor. The CO_2 production curves in the other

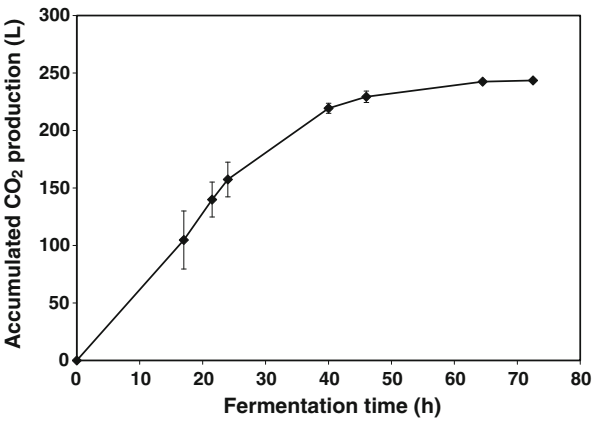

Fig. 1 Production of carbon dioxide in 7.5-l fermentor using Thoroughbred hulled barley at 30% initial total solids

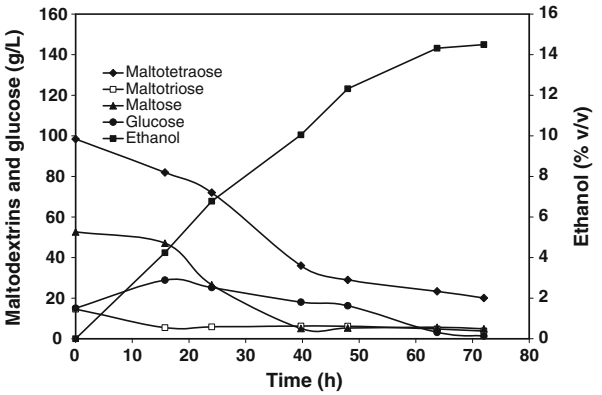

Fig. 2 Concentration profiles of maltodextrins, glucose, and ethanol in 70-l fermentor using Thoroughbred hulled barley at 30% initial total solids

experiments follow a similar pattern (results not shown). The time curves of CO_2 production indicate that the fermentations in the 7.5-l fermentor were practically completed at around 60–65 h.

The concentration profiles of maltodextrins and glucose in the case of the hulled barley in the 70-l fermentor are shown in Fig. 2. It should be pointed out that the HPLC column used for sample analysis could not separate maltodextrins and cellodextrins. However, because of the much smaller quantities of β-glucans compared to those of starch, it could be assumed that the contributions of cellodextrins to the peaks on the chromatograms were insignificant. Thus, for simplicity, the terms maltodextrins, maltotetraose, maltotriose, and maltose were used to describe the corresponding peaks. The results show a highly efficient SSF process. Glucose concentration stayed below 30 g/l most of the time. It increased only during the first 15 h of the fermentation then continuously decreased. Maltose, maltotriose, and maltotetraose all decreased with time. At the end of the fermentation, maltose, maltotriose, and glucose were all close to exhaustion. Only the maltotetraose peak remained relatively high at about 20 g/l, but this peak also may contain oligosaccharides larger than a degree of polymerization of 4 (DP-4) as well as other soluble materials excluded from the column at this retention time, so the actual amount of maltotetraose present may be considerably smaller than that indicated in Fig 2. Ethanol production and carbon dioxide production are shown in Fig. 3. The two curves are closely in parallel to each other. The calculated ethanol/CO_2 molar ratio is equal to one as shown in Table 2, again indicating that

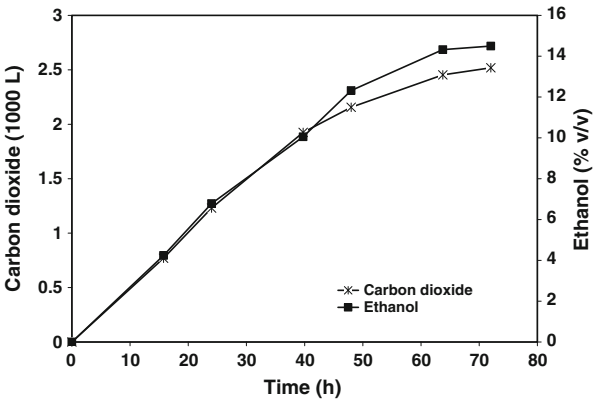

Fig. 3 Time course of carbon dioxide and ethanol production in 70-l fermentor using Thoroughbred hulled barley at 30% initial total solids

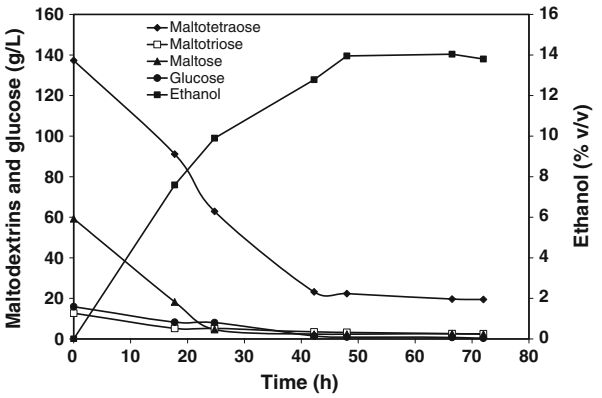

Fig. 4 Concentration profiles of maltodextrins, glucose, and ethanol in 70-l fermentor using Eve hull-less barley at 28.15% initial total solids

carbon dioxide production indeed can be used to follow the progress of ethanol fermentation. The final ethanol concentration was 14.5% (*v*/*v*) and the yield was 86.1% of theoretical value.

The concentration profiles of maltodextrins, glucose, and ethanol in the case of the hull-less barley in the 70-l fermentor are shown in Fig. 4. The CO_2 data were not available in this experiment due to the accidental breaking of the gas line between the fermentor and the gas meter in the middle of the experiment, which negated all CO_2 production measurements. Similar to the case of the hulled barley, a highly efficient SSF process was observed as indicated by the continuous decreases of maltodextrins and glucose. The final ethanol concentration was 14.0% (*v*/*v*) and the yield was 83.4% of theoretical value, which were slightly lower than those observed in the 7.5-l fermentor.

The concentration profiles of maltodextrins, glucose, and ethanol, plus the total CO_2 production in one of the two duplicated runs using the hull-less barley in the 300-l fermentor are shown in Fig. 5. Again, a highly efficient SSF process was observed as indicated by the continuous decreases of maltodextrins and glucose. The average final ethanol concentration was 12.86% (*v*/*v*), and the average ethanol yield was only 78.1% of the theoretical value. This, along with the low ethanol/CO_2 molar ratio of 0.87 as shown in Table 2, suggests that the condenser on top of the 300-l fermentor may have allowed some ethanol to escape with the fermentor exhaust. In fact, if the theoretical ethanol yield was assumed to be 85%, the calculated ethanol/CO_2 molar ratio would be very close to unity.

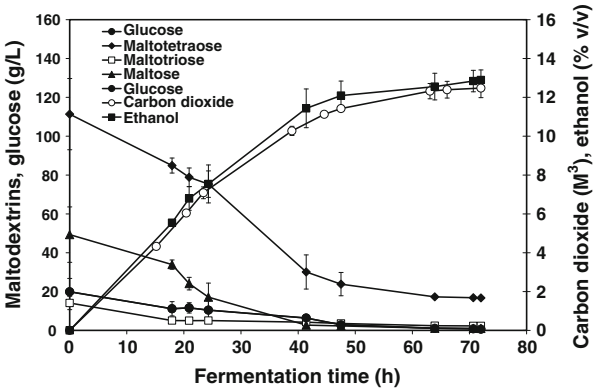

Fig. 5 Progress of fermentation in 300-l fermentor using Eve hull-less barley at 28.15% initial total solids

Table 3 Summary of mass balance calculations

	Fermentor size		
	7.5 l	70 l	300 l
(g C in identified products/g C converted)×100%			
Thoroughbred	94.0±2.4	92.9	90.2±1.1
Eve—28.15% solids	97.6	N/A	
Eve—30% solids	93.5		

A mass balance also was performed on the results of the fermentation experiments. In this mass balance, the total grams of carbon in all of the identified products, which included ethanol, carbon dioxide, glycerol, succinic acid, lactic acid, and acetic acid, is compared to the total grams of carbon converted, which included the total grams of carbon in the starch and β-glucans contained in the barley feedstock less the total grams of carbon in the residual maltodextrins and glucose. As stated earlier, the maltotetraose peak actually contained all soluble oligosaccharides having four or more glucose units. For our calculations, however, we assumed that this peak only contained maltotetraose and the amount of mass in the peak was estimated using this assumption. The results of the mass balance are summarized in Table 3. The values of the ratio (grams of C in identified products per gram of C converted) vary from 93% to 98% for the results obtained in the 7.5- and 70-l fermentors. These calculated values are consistent with our previous observations that typically about 5% of the glucose consumed would be converted to the *S. cerevisiae* cell mass in ethanol fermentation (unpublished results). The relatively low value of 90% for the ratio (grams of C in identified products per gram of C converted) in the case of the 300-l fermentor probably can be attributed to the loss of some of the ethanol produced by evaporation as discussed previously.

The compositions of the DDGS co-products are summarized in Table 4. As expected, the hulled barley DDGS had higher fiber but lower protein contents compared to the hull-less barley DDGS. The average protein and NDF contents in corn DDGS have been reported to be 30.92% and 44.73%, respectively [17]. Thus, the hulled barley DDGS had protein and

Table 4 Compositions of the DDGS co-products

DDGS components	Quantity of DDGS components (% dry basis)				
	7.5-l TB	7.5-l Eve	70-l TB	70-l Eve	300-l Eve
NDF	33.34±1.56	20.99±2.96	37.93	25.77	28.81±2.39
Protein	24.32±1.57	34.42±1.10	21.76	33.14	33.25±0.09
NDICP	1.66±0.08	3.31±1.45	4.76	7.48	12.18±1.88
Crude fat	4.49±1.01	6.51±0.31	5.5	7.83	6.38±1.25
Starch	1.72±0.58	1.53±0.36	1.43	1.39	1.37±0.56
β-Glucans	0.16±0.05	0.31±0.08	0.45	0.23	0.15±0.06
Ash	5.12±0.88	4.48±0.12	5.98	4.72	4.88±0.41
NFC	37.79±2.25	36.91±2.23	33.59	36.02	38.87±1.27
Phytic acid	0.22	0.42	0.16	0.28	0.09

NDF contents both lower than those in corn DDGS whereas the hull-less barley DDGS had protein contents matching those in corn DDGS but lower NDF contents. The composition of DDGS from a given barley variety would be expected to be relatively constant regardless of the scale of the fermentation used to produce it. In these experiments, this is generally the case except for values of NDF and NDICP. For instance, for Eve barley, the values for crude protein, crude fat, residual starch, β-glucans, NFC, and phytic acid in DDGS from all three fermentor sizes are quite similar in Table 4. However, the values for both NDICP and NDF for Eve are proportionally larger for the larger scale fermentations. This is likely due to differences in the way that the DDGS samples were processed for the different fermentors. For the 7.5-l fermentations, ethanol was not removed from the beer by boiling and the whole stillage was dried at 70 °C. For the larger fermentations, ethanol was removed from the whole stillage by boiling, followed by drying in a tumbling dryer at 80 °C. DDGS samples from the larger fermentors therefore experienced higher and extended temperatures, which denatured protein, leading to lower solubility (higher NDICP values) and corresponding higher NDF values. Thus, it is expected that DDGS from Eve barley produced at any scale should be similar to that shown here for all components except NDF and NDICP, which will vary depending upon downstream processing conditions. The β-glucan levels in all DDGS samples were extremely low, making them suitable for use in feeds for all animals, including ruminants and monogastric animals that cannot tolerate high levels of β-glucans, such as poultry. The phytic acid levels in all of the DDGS were also low and were below the reported values for some animal feeds, which are 8.85, 10.80, and 9.02 mg/g dry matter for pigs, sow, and hen feeds, respectively [18].

Conclusion

It has been demonstrated that the EDGE process, which was developed using a shake flask model for Thoroughbred, a winter hulled barley, could also be used for Eve, a winter hull-less barley. It was shown that the process was scalable in fermentors up to 300-l volume for both barley varieties. The SSF process was highly efficient as indicated by low levels of glucose throughout the course of the fermentation. Final ethanol concentrations of 14% (*v*/*v*) were achieved for initial total solids of 28–30%, which gave an ethanol yield of 83–87% of the theoretical values. The DDGS co-product contained very low levels of β-glucans and thus was suitable for use in feed formulations for all animal species.

Acknowledgments The authors would like to express their sincere thanks to Gerard Senske of the ERRC, who performed the experiments. The invaluable assistance of Mike Pepsin of Genencor and Robert Moreau, Michael Powell, Mike Kurantz, and Jennifer Thomas of the ERRC in sample analysis also is sincerely appreciated. This work was partly funded by Cooperative Research and Development Agreement # 58-3K95-6-1155-M between Genencor, a Danisco Division, and ARS-USDA.

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