Effects of hydrodynamic cavitation on dry mill corn ethanol production

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A B S T R A C T
In this study, the ability of hydrodynamic cavitation pretreatment (HC) to increase ethanol yields at dry mill corn ethanol plants was investigated at pilot scale. Experiments were conducted at four HC energy densities, with and without jet cooking pretreatment and at a range of alpha-amylase (AA) doses. Corn slurries treated with HC had reduced particle sizes and significantly higher soluble saccharides. After simultaneous saccharification and fermentation (SSF), ethanol yields were 2.6% greater than in uncavitated controls. Jet cooking alone decreased ethanol yield compared to cavitated and control treatments. HC combined with jet cooking resulted in ethanol yields similar to controls. At alpha-amylase dosages 60% lower than those used at commercial scale plants (0.40% w enzyme/w dry corn), there was only a slight, insignificant reduction in ethanol yield. The net energy yield of HC was 47 kWh in additional ethanol for each kWh used for HC. In conclusion, HC improved the solubilization of saccharides and ethanol yield from corn, and had a positive net energy yield, thereby improving the efficiency of dry mill corn ethanol production from both an economic and environmental standpoint.

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1. Introduction

In 2014, the United States produced 54 billion liters of corn ethanol biofuel [1] primarily using the dry-grind (DG) or dry mill ethanol process [2]. In this process the main steps are hammer milling, slurry preparation, cooking, liquefaction, saccharification, fermentation, distillation, and co-product recovery [3,4]. The process generates three main products: ethanol, distillers dry grains with solubles (DDGS) and carbon dioxide (CO2) [5,6].

A significant amount of starch remains unconverted to ethanol during this process. For example, the amount of starch in DDGS ranges from 5% to 7% on a dry weight basis [3,5,7,8]. This is primarily because the residual starch is inaccessible, incompletely solubilized or bound to cell wall structures or other cell components. In addition to this, some starch is likely converted to Maillard products during high temperature jet cooking and DDGS drying and not accounted for in DDGS analysis [9–11]. This led Schell et al. [12], to conclude that liberating this starch could increase ethanol yields at DG ethanol plants.

Studies have shown that reducing the particle size of corn grain by milling significantly boosts ethanol yield at dry mill corn ethanol plants. This is likely due to improvements in starch accessibility. For example, Kelsall and Lyons [13] reported a 7.5% increase in ethanol yield using corn ground to 4 mm versus 8 mm. Naidu et al. [4], reported a 22% increase in ethanol yield when a 0.5 mm versus a 5 mm sieve screen was used for corn milling. Unfortunately, the use of dry milling to reduce the mean particle size of corn below 1 mm becomes increasingly energy intensive, limiting this approach. For example, Wondra et al. [14], reported that milling corn to a 0.4 mm versus a 1.02 mm mean geometric diameter required three times more electrical energy and substantially more capital investment.

Another approach that has been used to reduce particle sizes in slurries is cavitation. Cavitation is the formation and collapse of bubbles or cavities in a liquid. This collapse generates powerful hydro-mechanical shear forces in the bulk liquid that disproportionately fracture large particles [15]. There are four types of cavitation: hydrodynamic cavitation (HC), ultrasonic cavitation (UC), optical cavitation (OC) and particle cavitation (PC). The last two are not generally applied at industrial scales due to their...
energy inefficiencies. However, the first two are quite commonly used in certain industries such as wastewater treatment, polymer chemistry, petroleum, nanotechnology, medical equipment, and biotechnology [16–19]. UC is a result of pressure variation in a liquid when ultrasound waves pass through it and HC is produced by pressure variations in a flowing liquid caused by velocity variations in the system [15].

Recently, a series of studies was conducted to evaluate the use of UC technology in dry grind ethanol production from corn [20–24]. These studies reported that UC generated micro pores in corn particles, significantly reduced particle size, and improved glucose release after corn slurry liquefaction and saccharification (from 7% to 38%) and ethanol yield by about 10% after SSF as compared to uncavitated-control samples. Net energy gain of UC treatment, defined as the chemical energy content of the additional glucose released and/or ethanol produced by UC divided by the input energy used for UC, exceeded 1 for the optimum UC treatments, which indicated that the use of UC was slightly energetically favorable. Studies have also reported that alpha-amylase and glucoamylase enzymes were not inactivated by UC as they were by jet cooking pretreatment [20–24]. So if UC were used in place of jet cooking, enzyme dosage and cost could be reduced substantially. A cost analysis comparing jet cooking to UC installed at commercial scale, showed a lower overall cost for UC treatment as compared to jet cooking over a 10-year timeframe, largely due to the lower energy requirements for UC as compared to jet cooking [23,24].

However, there are disadvantages with UC. For example, UC is reasonably easy to test at lab scale but is not particularly scalable, reliable or energy efficient at larger commercial scales [16,19,25]. This is because acoustic energy cannot be effectively transmitted into large process volumes since UC energy intensity decreases exponentially as a function of the distance from the sound source. Therefore, complex designs are required in order to establish cavi-
tation zones and transfer UC energy to large volumes [19]. This has limited the use of UC at commercial scales.

Hydrodynamic cavitation (HC), in contrast, is more easily scalable but difficult to study at small lab scales. HC may provide similar benefits, require less energy, and be more easily scaled and more reliable as compared to UC [16,25]. HC can be generated by pumping a liquid through a constriction [19].

Although HC has been applied in a variety of industries [18], to date there is only one study [26] on HC applied to ethanol production (to our knowledge). An HC system installed at a commercial scale dry mill ethanol plant (production capacity 379 million liter of ethanol per year) was reported to reduce the particle size distribution, lead to qualitative changes in cell structure, reduce total solids by 3%, increase total sugars in solution after liquefaction, and enhance ethanol yield by 2.2% as compared to uncavitated-control samples [26]. It was also demonstrated that the energy return of HC in the form of ethanol was 16 times greater than the energy expended to generate cavitation (as compared to slightly greater than 1 for UC). However applications of UC at a range of energy densities, direct comparison of jet cooking and HC, and an assessment of the extent of enzyme inactivation by jet cooking versus HC, were not done in this previous study.

The purpose of this study was to further evaluate the effects of HC on DG ethanol production. Tests were conducted at pilot scale under conditions that mimic full scale corn ethanol production conditions. The effects of HC at a range of energy densities on particle size distribution, starch extraction, ethanol yield, and energy gain in a DG process were determined. HC was also tested with and without jet cooking, and with reduced levels of alpha amylase than those typically used in the DG process.

2. Materials and methods

2.1. Feedstocks, chemicals, enzymes and yeast

No. 2 yellow-dent corn was obtained from The Ohio State University/Ohio Agricultural Research and Develop Center (OSU/OARDC) feed mill and the National Corn-to-Ethanol Research Center (NCERC), located in Wooster, Ohio and Edwardsville, Illi-
nois, respectively. Corn provided by OSU/OARDC was used during the effect of HC on starch release experiment (Section 2.4.1), and corn obtained from NCERC was used during the effect of HC on ethanol yield experiments (Section 2.4.2). The moisture content of the corn was 12.6% w/w.

Two types of alpha-amylase enzymes and one type of glu-
coamylase were used. The alpha-amylases were Liquozyme® SC DS (Novozyme, Franklinton, NC) and Spezyme® Xtra (Genencor® International (now DuPontTM Genencor®), Palo Alto, CA), and the glucoamylase was G-zyme® 480 (Genencor® International (now DuPontTM Genencor®), Palo Alto, CA). The Liquozyme® SC DS was used in experiments on the effect of HC on starch release (Sec-

To prepare SSF media (Section 2.4.2), lactrol antibiotic (PhibroChem), yeast nutrient (AYF177, ethanol Technology), commercial grade urea and yeast (Ethanol Red; Fermentis, Marcy-
en-Baroeul, France) were used.

HPLC standard chemicals including maltotriose, maltose, glu-
cose, lactic acid, glycerol, and acetic acid were purchased from Fisher Chemicals and Sigma–Aldrich. 200 proof ethanol and DP4+ standards were obtained from Pharmoc-Aeper and Grain Process-
ing Corp., respectively. These standards were used for saccharides, ethanol and byproducts quantification using HPLC.

Triton X-100, 72% H2SO4 at 72%, and DMSO were purchased from Sigma–Aldrich, while MOPS, sodium acetate and acetic acid were purchased from Fisher Chemicals.

2.2. Pilot scale HC system

A pilot scale HC system was built and used to test the effects of HC on starch extraction, particle size distribution and ethanol production from corn slurry. The system was designed to mimic the unit operations in a commercial scale dry mill ethanol plant. It included a 379L heat-jacketed vessel able to maintain slurry tempera-
tures of from 82°C to 94°C using a hot oil heater. A low-shear impeller mixer within the vessel maintained particle suspension and agitation in the slurry. From this vessel, the corn mash was pumped directly to the inlet of a 74.6 kW centrifugal pump. This pump was capable of maintaining pressures as high as 3 MPa at 68.1 L/min. The corn slurry was then pumped directly through a HC device.

The cavitation unit was constructed based on US patents 5,937,906; 5,971,601; and 6,035,897. Fig. 1 shows a schematic drawing of the HC device used in this study. The unit comprised two flow-through channels separated by a diaphragm with multiple ori-

sources which produced a flow constriction. Hydrodynamic flow to the inlet channel of the unit was provided by the centrifugal pump. The minimum velocity for the flow was between 1 m/s to 10 m/s. The diaphragm generated a pressure difference between the two channels which accelerated the flow through the orifices creating a cavitation zone that became saturated with cavitation bubbles. This zone was specifically created when the slurry passed through the unit at a velocity capable of generating cavitation bubbles (at least 16 m/s). Then the flow passed to the outlet channel where the static pressure increased to initiate bubble collapse. Here the energy accumulated in the bubbles was transferred to the flowing
material. The slurry was maintained in this zone for approximately 0.5 s.

Pressure was monitored using standard diaphragm gauges and differential pressure transmitters situated prior to, and after the cavitation unit. The gauge prior to HC unit showed the pressure applied to corn slurry for cavitation by the pump, while the gauge after the HC unit displayed the dynamic head pressure of the downstream flow. A sampling port was situated after the cavitation unit. A diverter valve after the cavitation unit provided the ability to either re-circulate the slurry stream or to direct it downstream for further processing (Fig. 2).

2.3. HC system parameters quantification

Effects of hydrodynamic cavitation on process parameters such as: temperature, pressure, cavitation energy density and flow rate; were determined by installing a hydrodynamic cavitation unit at NCERC (Fig. 3A and B). Temperature probes, flow meters and differential pressure transmitters were located before the cavitation pump, between the cavitation pump and the cavitation unit, and after the cavitation unit. In the hydrodynamic cavitation system, the slurry was conveyed to a cavitation unit using a centrifugal pump. The pump accelerated the slurry creating a pressure difference in the cavitation unit in order to generate cavitation zones (Fig. 1).

Changes in the hydrodynamic energy density were calculated using pressure change values before and after HC and the density of the corn slurry before cavitation. Experiments were conducted at three HC energy densities (313, 940 and 1567 J/kg slurry; labeled as Cav2A, Cav2B, and Cav2D, respectively). The HC system was started and data was recorded for approximately 200 s using a Yokogawa Daqstation DX200.

2.4. Experimental procedures

2.4.1. Effect of HC on starch release

Corn slurry was prepared by placing 268 L of DI water in the 379-L heat-jacketed vessel of the HC system and heating to 96 °C with constant stirring at 2200 rpm. Then 134 kg (dry weight) of hammer milled and sieved 7/64 in-screen (2.74 mm perforation size) No. 2 yellow-dent corn provided by OSU/OARDC and 22 g of alpha-amylase enzyme Liquozyme® SC DS were added to the vessel (Fig. 2). After the addition of corn and enzyme, the slurry was mixed for 20 min and pH and temperature were measured to be 5.9 and 80 °C, respectively. The mash was then pumped through the HC unit. Four different energy densities were tested: 627, 940, 1254, 1567 J/kg slurry, plus control (0 J/kg slurry). Four samples at each level of energy density tested were collected in 500 mL Nalge plastic bottles and analyzed for particle size distribution and total and soluble starch.

2.4.2. Effect of HC on ethanol yield

For this experiment, the pilot scale HC system was installed and tested at a larger comprehensive pilot scale ethanol production plant located at the US NCERC facility in Edwardsville, Illinois. This system was integrated into the existing dry mill corn ethanol pilot plant at NCERC which closely mimics the configuration of a commercial scale ICM dry mill ethanol plant (Fig. 3A and B).

Whole kernel No.2 yellow-dent corn provided by NCERC was pneumatically conveyed from storage bins to a destoner and then to a scalper/screen to remove large, heavy, and fines particles. The cleaned corn was fed at a controlled rate of 442.2 kg/h into a hammer mill, sieved through a 7/64 in-screen (2.74 mm perforation size), and transferred to a receiver placed above the slurry mixer. The ground corn was mixed with hot water fed at a rate of 13.5 L/h in a 379 L tank (tank 1) with a working volume of 341 L, resulting in a slurry with a 30.9% w/w solids concentration. Forty percent of the total alpha-amylase Spezyme® Xtra dose used at commercial scale ethanol production (the total dose of alpha-amylase at commercial scale is 0.04% w/w of dry corn) was added at a rate of 70 g/h. Temperature, pH and level of the slurry were constantly monitored by in-line instrumentation. The slurry was heated to a target temperature of 77 °C using a shell-and-tube heat exchanger. The pH was maintained at 5.7–6.0 using automated addition of sulfuric acid or aqueous ammonia (Fig. 3A and B).

The effects of HC at three energy density levels 313, 940, and 1567 J/kg slurry (labelled Cav2A, Cav2B, and Cav2C, respectively) as compared to jet cooking (Jet) and no pretreatment (Ctrl), along with three different alpha-amylase doses (0.016, 0.028 and 0.040% w/w of dry corn, named 40%, 70% and 100%, respectively) were determined using process configuration 1 (Fig. 3A). The parameters measured were ethanol yield, saccharides and byproducts from corn slurry after liquefaction and SSF.

For this experiment, the 227 L of slurry from tank 1 (working volume 379 L) was pumped to tank 2 (working volume 379 L), using a positive displacement pump at a rate of 17 ± 0.8 L/min. After steady-state was achieved, the same rate was used to discharge tank 2. When steady state was reached for the charge and discharge rates in tanks 1 and 2, a three-way valve after tank 2 was used to divert the flow through the cavitation unit, through a jet cooler, or to sample port 1.

For HC treatments, the discharge of the slurry from tank 2 was pumped through the cavitation system using a high-pressure centrifugal pump at flow rates from 26.5 L/min to 56.8 L/min. Three different energy densities (313, 940 and 1567 J/kg slurry) were tested. Samples after HC were collected from sample port 2 (Fig. 3A). For controls, the slurry in tank 2 was diverted to sample port 1.
(Fig. 3A). For Jet Cook treatments, slurry in tank 2 was diverted to the jet cooker (Hydro-Thermal model M103AS). The slurry in the jet cooker was maintained under pressure at elevated temperature (106 °C) while it was pumped through a 79.5 L hold tube and later discharged through a flash vessel into tank 3 (1893 L), where the pressure was reduced to atmospheric. Cavitated and jet cooked samples were collected from sample port 6 (Fig. 3B).

Slurry samples produced by process configurations 1 and 2 (Fig. 3A and B) were transferred to 1 L bottles and mixed with an overhead agitator to ensure appropriate mixing. 160 g of slurry were then transferred using a peristaltic pump to tared and sterilized 250 mL Erlenmeyer flasks.

Samples obtained from process configuration 1 were dosed with 40%, 70% and 100% of the alpha-amylase Spezyme® Xtra of a total dose of 0.04% w enzyme/w dry corn in two doses. The first dose of 0.016% w alpha-amylase enzyme/w dry corn (40% of 0.04% w enzyme/w dry corn) was added during slurry preparation to start liquefaction as described above. The second dose was added after sampling. The second dosages were 6% w enzyme/w dry corn (total of 40% of 0.04% w enzyme/w dry corn), 0.012% w enzyme/w dry corn (total of 70% of 0.04% w enzyme/w dry corn) and 0.024% w enzyme/w dry corn (total of 100% of 0.04% w enzyme/w dry corn).

Slurry samples collected from process configuration 2 were amended with 0.04% w enzyme/w dry corn Spezyme® Xtra. The enzyme addition was done in two stages. 40% of the 0.04% w
enzyme/w of dry corn was added during slurry preparation and 60% of the 0.04% w enzyme/w dry corn was added after sampling.

After the second dose of alpha-amylase enzyme, flasks were liquefied by heating to 82 °C in a water bath and shaking at 135 rpm for 1 h. Flasks were then cooled to 35 °C using a cold water bath and agitation at 150 rpm, and then transferred to an incubator shaker at 32 °C and 150 rpm. The pH was adjusted to 5.2 using 5 M H2SO4 and antibiotic Lactrol was added to a final concentration of 0.5 mg/kg of slurry.

To initiate SSF, Glucoamylase enzyme G-zyme® 480 (0.10% w/w corn), urea (500 mg/kg of slurry) and yeast nutrients (1500 mg/kg corn) were added to the flasks. A yeast suspension of 0.10 g/mL was prepared in a 250 mL sterile flask. The suspension was incubated at 40 °C for 20 min. Each flask was then inoculated with 352 µL of the yeast suspension to attain an initial concentration of 2 × 10^7 cells/mL. When all the medium components were added, the weight of the flasks was recorded and gas traps were inserted. The flasks were incubated at 32 °C, 150 rpm for 60 h in an incubator shaker (Sartorius, Certomat BS-1).

After SSF, the weights of the flasks were recorded, samples from each flask were acidified to pH 2 by addition of 5 M H2SO4, and ethanol and byproducts were quantified using HPLC.

2.5. Analytical methods

2.5.1. Particle size analysis

Particle size distribution in corn slurry samples was measured using a Horiba LA-950 (Horiba Inc.) laser diffraction particle size analyzer. The particle size distribution was measured within 30 min of sample collection. Samples (~30 mL) were transferred to a 50 mL centrifuge tube and the pH was reduced to 3.5 by the addition of 2 M H2SO4 which inactivated the alpha amylase. One g samples were dispersed in DI water in the analyzer and 50 µL of surfactant (0.1% Triton X-100 in DDI water) was added to prevent particle agglomeration. Particle size distribution analysis was performed using LA-950 software provided with the device.

2.5.2. Starch quantification

The amounts of starch in corn slurry liquid and solid fractions were determined using a Megazyme total starch assay (Megazyme International Ireland, Bary Business Park, Bary, Co., Wicklow, Ireland) with some modifications (AOAC Method 996.11). Samples of liquefied corn slurry (30 mL) were transferred to 50 mL centrifuge tubes and then to an ice bath for 10 min to cool and prevent further enzyme hydrolysis. Samples were then centrifuged at 4000 rpm for 15 min at 18 °C. The supernatant (liquid fraction) was decanted and stored at 3 °C for analysis as described below.

The solid fraction was washed twice with DI water and dried at 40 °C to a constant weight. The dry solids were then ground and sieved through a US standard mesh N. 60 screen. A 100 mg sample of the sieved solids was transferred to a 50 mL centrifuge tube and 5 mL of 80% ethanol was added. The tubes were incubated in a water bath at 80–85 °C for 5 min and then removed from the bath and 5 mL ethanol 80% was added. Tubes were then centrifuged at 4000 rpm for 10 min at room temperature, the supernatant was discarded and the pellet was resuspended in 10 mL of 80% ethanol. The tubes were centrifuged again at 4000 rpm for 10 min at room temperature and the supernatant was discarded.

For liquid fractions, 0.7 mL of the liquid fraction was mixed with 0.2 mL of 80% ethanol in 50 mL centrifuge tubes. Thereafter both solid (without soluble sugars) and liquid fraction were processed as follows.

Two mL of DMSO was added to the centrifuge tubes. The tubes were immediately transferred to boiling water for 5 min and 3 mL of alpha-amylase from total starch Megazyme kit, diluted in MOPS buffer pH 7.0 (1:29), was added. Tubes were incubated for 6 min in
boiling water with stirring every 2 min and then they were transferred to a 50 °C water bath for 3 min. 4 mL of 200 mM sodium acetate buffer pH 4.5 and 0.1 mL of amylglucosidase from the kit were added. Next the tubes were incubated for 30 min and stirred every 5 min. After hydrolysis, tubes were transferred to an ice bath for 10 min and stored at 4 °C to inactivate the enzymes. Samples were analyzed using HPLC. Each batch of samples were run with a set of controls from the Megazyme resistant starch controls kit.

2.5.3. Saccharides, ethanol and byproducts quantification Samples for HPLC were filtered through 0.45 μm syringe filters into 2 mL HPLC vials. HPLC analysis was conducted using Agilent 1200 and/or Shimadzu systems with RID detectors. In both systems the mobile phase used was 0.005 mol/L H2SO4 at a flow rate of 0.6 mL/min and the injection volume was 10 μL. The Agilent 1200 system was used during the effect of HC on starch release experiment (Section 2.5.1), and the Shimadzu system was employed in the effect of HC on ethanol yield experiments (Section 2.5.2). A Rezex ROA-Oligoacid H+ (8%) column (Phenomenex®, Torrance, CA) and security guard column AJO-4492 (Phenomenex® Torrance, CA) at 80 °C were used in the Agilent HPLC. An HPX-87H column and micro-guard cartridge (BioRad® Hercules, CA) at 60 °C were used in the Shimadzu HPLC. The systems were calibrated using HPLC grade standards of DP4+, maltotriose (DP3), maltose (DP2), glucose (where “DPn” represent glucose oligomers with “n” subunits), lactic acid, glycerol, acetic acid, and 200 proof ethanol. Linear calibration curves were calculated using six calibration points. All had R2 values greater than 0.99.

2.6. Statistical analysis

All statistical analyses were conducted using JMP® software with a level of significance of 5%.

3. Results and discussion

3.1. Effect of HC system on process temperature

During cavitation, temperature increases of 3.2 °C, 6.2 °C and 8.4 °C occurred in cavitation treatments Cav2A, Cav2B, and Cav2C, respectively (Table 1). This was due to flow acceleration of the slurry in the centrifugal pump (Table 1) and not in the HC device and was proportional to the flow rate used to generate the different cavitation energies (R2 = 0.99). These short term temperature increases are unlikely to have affected starch release and SSA. In a commercial scale HC system, temperature increases of 1–2 °C were observed due to the HC device at an energy density of 1560 J/kg (personal communication, Fred Clarke, Arisdyne, Inc.).

3.2. Effect of HC system on corn slurry particle size

The effect of HC on particle size distribution was measured at energy densities of 0, 627, 940, 1254, and 1567 J/kg slurry (labeled DP4+, DP3, maltose, and glucose were expressed as unfermented total glucose (UFTG). UFTG was calculated based on the molar ratio of the oligosaccharide to the monomer using the following equation. UFTG = 1.11 × DP4 + 1.07 × DP3 + 1.05 × Maltose + Glucose.
as Control (Ctrl), Cav1A, Cav1B, Cav1C, and Cav1D, respectively). The sizes of corn slurry particles were noticeably different for the control, and cavitated samples at all HC energy densities (Fig. 4A). The particle size distributions showed two main peaks. In control samples the peaks were located at particle diameters of 890 μm and 12 μm. These larger particles are commonly referred to as “corn meal” while the smaller sized particles are likely to be released starch granules [27,28]. The same two peak sizes were also observed in the cavitated samples. However the large particle size peak was diminished in size and broadened while the starch granule peak increased in proportion to the energy density. At the greatest energy density (Cav1D), both peaks had the same height. The increase in the height of the smallest particle size peak as the energy density increased, likely was the result of the release of individual starch granules into the bulk liquid phase.

These results are similar to the particle size reductions reported by Ramirez-Cadavid et al. [26], Khanal et al. [28], and Nitayavardhana et al. [29], in HC and UC treated slurries of corn or cassava, respectively.

### 3.3. Effect of HC on starch release

The effect of HC on starch release was measured in the same set of samples described above in Section 3.2. The total amount of starch in the initial slurry was 0.62 ± 0.01 mg starch/mg of dry corn. The amount of starch released due to cavitation increased while the residual starch decreased as a function of the cavitation energy density (Fig. 4B). The greatest starch release occurred at the maximum cavitation energy density (Cav1D), with a yield of 0.48 mg glucose/mg of dry corn (0.43 mg starch/mg of dry corn) as compared to 0.43 mg/mg of dry corn (0.39 mg starch/mg of corn) for the control treatment representing an increase of 11%. The starch remaining in the solid phase was 0.20 mg per mg of dry corn (0.18 mg starch/mg of corn) in the Cav1D treatment as compared to 0.26 mg per mg of dry corn (0.18 mg starch/mg of corn) in the uncavitated control. There was not a statistical difference among the three highest cavitation energy density treatments Cav1B, Cav1C and Cav1D (p-value > 0.05). But all three of these treatments were significantly greater than the control and the lowest energy density cavitation treatment Cav1A (p-value < 0.05). It is important to note that this is an intermediate step in the process and that even at the highest energy density tested, the starch release represented just 77% of the total starch present in the corn. Additional starch release would be expected to occur during the subsequent processing steps of liquefaction and SSF.

These results are consistent with previous research on cavitation showing an increase in oligosaccharide and disaccharide concentrations after cavitation during corn ethanol production. Ramirez-Cadavid et al. [26], reported an increase of 2–4% in total sugars after hydrodynamic cavitation pretreatment at a commercial scale DC ethanol plant. Likewise, Khanal et al. [28], Nityavardhana et al. [29], Montalbo-Lomboy et al. [20], and Nickolic et al. [21], found significant increases in starch release after the application of UC.

### 3.4. Effect of HC on ethanol yield and fermentation byproducts

#### 3.4.1. HC energy density, jet cooking and alpha amylase dose

A factorial 5 × 3 treatment design was used to study the effects of two factors; pretreatment (cavitation at four energy densities, and jet cooking) and enzyme dosage, on ethanol yield. The factors were grouped into 15 treatments (5 pretreatments and 3 enzyme doses) with three replicates per treatment.

These results indicated that there was not a significant effect (p < 0.05) of any of the 15 treatments on ethanol yield when evaluated by multi-factor ANOVA. All the treatments grouped into one homogeneous group at a p < 0.05 (Table 2). The treatments also did not have a significant effect on UFTG, lactic acid or acetic acid (p-value > 0.05); but did have a significant effect on glycerol and CO2 (p-value < 0.05) (Table 2).

Since the two factor analysis showed no effect, treatments with the same enzyme dosage, or the same pretreatment, were grouped together to give more replicates (n = 15 and n = 9 for alpha-amylase and pretreatment, respectively) to better assess whether alpha-amylase dose or the individual pretreatments had significant impacts on ethanol yield. One-factor ANOVA on pooled means showed that there were significantly different ethanol yields for the different levels of pretreatment (p-value < 0.05) (Table 3). Significantly higher ethanol yields were observed at the two highest HC energy densities as compared to the lowest energy density and the control by Tukey–Kramer’s HSD test. The increases in ethanol yield were 2.6% and 1.8% higher in Cav2C and Cav2B, respectively, as compared to the uncavitated control. The lowest HC energy density (Cav2A), jet cook and control treatments were not significantly different from each other (Table 3). There was no difference in the yield of most byproducts in the different treatments with the excep-

### Table 4

Effects of alpha amylase dose on ethanol yield, UFTG and byproducts using process configuration 1. One-factor ANOVA and Tukey–Kramer’s HSD test on pooled pretreatment means.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ethanol 3%/w dry corn</th>
<th>UFTG 3%/w dry corn</th>
<th>Lactic acid 3%/w dry corn</th>
<th>Glycerol 3%/w dry corn</th>
<th>Acetic acid 3%/w dry corn</th>
<th>CO2 3%/w dry corn</th>
</tr>
</thead>
<tbody>
<tr>
<td>40%</td>
<td>35.20 ± 0.02 a</td>
<td>2.25 ± 0.21 a</td>
<td>0.11 ± 0.02 a</td>
<td>2.52 ± 0.06 a</td>
<td>0.14 ± 0.01 a</td>
<td>36.24 ± 0.82 a</td>
</tr>
<tr>
<td>70%</td>
<td>35.32 ± 0.02 a</td>
<td>2.33 ± 0.09 a</td>
<td>0.10 ± 0.01 a</td>
<td>2.53 ± 0.08 a</td>
<td>0.14 ± 0.01 a</td>
<td>36.23 ± 0.71 a</td>
</tr>
<tr>
<td>100%</td>
<td>35.47 ± 0.07 a</td>
<td>2.33 ± 0.06 a</td>
<td>0.11 ± 0.01 a</td>
<td>2.54 ± 0.09 a</td>
<td>0.14 ± 0.01 a</td>
<td>36.41 ± 0.72 a</td>
</tr>
</tbody>
</table>

Values are means (n = 15) plus or minus one standard deviation. ANOVA was applied for each test at each compound and Tukey–Kramer’s HSD test was conducted to identify differences among treatments. The same letter in each compound for each test denotes a homogenous group at level of significance of 5%.
tion of final UFTG concentration which was significantly lower in the jet cook treatment (Table 3).

The increases in ethanol yield due to cavitation can be attributed to an increase in available sugars as a result of HC. As shown in Section 3.3, cavitation increased the soluble glucose equivalents (starch, polysaccharides, oligosaccharides and glucose monomers) which would result in more glucose available for fermentation during SSF. The increases in glucose equivalents and ethanol yield are similar to those observed by Ramirez-Cadavid et al. [26], at a commercial scale ethanol facility using high energy density HC.

The lower concentrations of residual sugars in the jet cook treatment indicate that some of these may have been transformed into Maillard products. Temperatures greater than 100 °C and pressures of approximately 1690 MPa during jet cooking pretreatment are known to generate these products [9–11]. These compounds are formed when reducing carbohydrates and amino groups react at high temperatures leading to a wide variety of often brown products that are recalcitrant to fermentation [9]. However, Maillard components were not analyzed in this study and further research must be conducted in order to assess the effect of jet cooking on their formation.

One-factor ANOVA on pooled means per level of alpha-amylase showed that although mean ethanol yield increased slightly with alpha-amylase dose it was not significant (p-value > 0.05) (Table 4). This suggests that the dose of 0.040% w enzyme/w of dry corn currently used at many DG ethanol plants may be reduced to 0.028 or 0.016% w enzyme/w of dry corn to reduce costs without affecting ethanol yield.

The effects of alpha-amylase dose on other products and by-products of fermentation (UFTG, lactic acid, glycerol, acetic acid and CO2) were also not significant (Table 4). This result further supports the idea that alpha-amylase dose may be reduced at commercial scale plants. There was no statistical difference in residual UFTG at the different enzyme dose levels either which indicates that higher doses of alpha-amylase did not generate additional fermentable sugars.

Lactic acid and acetic acid are compounds commonly produced during corn ethanol fermentation by contaminating bacteria. They can inhibit yeast fermentation at concentrations greater than 4 mg/mL and 25 mg/mL, respectively [26]. The average concentrations of lactic acid and acetic acid among the treatments in this study were 0.041 mg/mL and 0.52 mg/mL, respectively (Tables 2–4). At these concentrations both were unlikely to inhibit SSF.

Glycerol is another byproduct produced during yeast fermentation in order to maintain redox balance and protect cells against high osmotic pressure [26]. Glycerol was similar among the treatments except for the jet cooked treatments which produced significantly more glycerol, although less than the amount that would interfere with fermentation (Tables 2 and 3). Increases in CO2 production were closely correlated with increases in ethanol production (correlation coefficient of 0.93) consistent with the increases in ethanol production observed due to cavitation (Table 3).

The net energy gain due to cavitation was determined by calculating the amount of chemical energy in the additional ethanol released by HC, and dividing this by the electrical energy expended for hydrodynamic cavitation (for pumping). The chemical energy content of ethanol (LHV) was assumed to be 28,865 kJ/kg and the analysis for net energy gain was based on the ethanol yield increase in treatment Cav2C. The relative net energy gain was 46.9 kJ chemical energy per kg HC energy expended. This result indicates that HC would not only increase ethanol yield but also improve the energy return on investment of the DG ethanol process. Previous research on the use of HC in a commercial scale ethanol plant has also shown a positive net energy gain due to HC. In that study, the energy in additional ethanol generated by cavitation at a commercial scale plant was 16 times greater that the energy expended [26].

3.4.2. HC and HC with jet cooking

Three treatments were compared to investigate synergistic effects of jet cooking and cavitation. All had an enzyme dose of 0.040% w/w of dry corn and HC energy density of 940 J/kg. The treatments were HC alone (Cav2B); HC plus jet cooking (Cav2Bjet) and an untreated control (Ctrl). The treatments were compared based on ethanol yield. However, UFTG, lactic acid, glycerol, acetic acid and CO2 production after liquefaction and SSF were also quantified. Three replicates were used for each treatment and the results were analyzed by ANOVA with a level of significance of 5% and Tukey–Kramer’s HSD test to identify homogeneous groups.

ANOVA results showed that there were significant differences in ethanol yield among the treatments (Table 5) (p-value < 0.05). Tukey–Kramer’s HSD test identified two homogeneous groups. One homogeneous group corresponded Cav2B which had a higher ethanol yield of 35.8% w/w dry corn than the other two treatments. This represented an increase of 2.3% in ethanol yield for Cav2B as compared to Ctrl treatment. The other group included treatments Ctrl and Cav2Bjet which had lower ethanol yields of 35.0% w/w dry corn and 35.3% w/w dry corn, respectively. The production of CO2 was proportional to the ethanol yield in all the treatments with a correlation coefficient of 0.98, and therefore the same homogeneous groups as those obtained for the ethanol yield were found for the analysis of CO2 production.

In summary, the results of this study show that HC at a high energy density significantly increases ethanol production as compared to non-cavitated controls, jet cooking or HC plus jet cooking treatment. This study expands and confirms previous results reported by Ramirez-Cadavid et al. [26]. The results suggest that HC improves by more than 2% the ethanol yield in the dry-grind process when used at energy densities greater than 940 J/kg slurry. They also indicate that applying HC by itself is a more effective pretreatment than a combination of HC and jet cooking or jet cooking alone. The results further suggest that the use of jet cooking in the dry-grind process should be reevaluated and that the alpha-amylase dosage typically used at commercial scale facilities may be reduced to improve process efficiency.

4. Conclusions

In conclusion, hydrodynamic cavitation (HC) applied under conditions that mimic those at commercial scale dry-grind ethanol plants, reduced corn slurry particle size and increased total saccharides and ethanol yield. HC ethanol yield was significantly greater as compared to jet cooked or uncavitated controls. Increases in the concentrations of sugars in solution and ethanol yield were proportional HC energy density. Alpha-amylase dose was reduced from 0.040% w/w to 0.016% w/w without a significant reduction in ethanol yield. The net energy yield of HC was 47 kJ of ethanol produced for every kJ of energy expended.

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References


