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Ultrasonic Pretreatment of Corn Slurry in Batch and Continuous Systems

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Abstract. *The effects of ultrasonication of corn slurry, on particle size distribution and enzymatic hydrolysis was studied for the dry-grind mill ethanol industry. Two independent ultrasonic experiments were conducted at a frequency of 20 kHz; in batch and continuous systems. The ground corn slurry (33% m/v) was pumped at flow rates 10-28 L/min in continuous flow experiments, and sonicated at constant amplitude (20$\mu m_{peak\text{-}to\text{-}peak(p\text{-}p)}$). Ultrasonic batch experiments were conducted at varying amplitudes of 192-320$\mu m_{p\text{-}p}$. After ultrasonication, Stargen™001 enzyme was added to the samples and a short 3h hydrolysis followed. The treated samples were found to yield 2-3 times more reducing sugar compared to the control (untreated) samples. In terms of energy density, the batch ultrasonic system was found to deliver 25-times more energy than the continuous flow systems. Although the experiments conducted in continuous system released less reducing sugar than the batch system, the continuous system was more energy efficient. The particle size of the sonicated corn slurry (both batch and continuous) was reduced relative to the controls (without treatment). The reduction of particle size was directly proportional to the energy input during sonication. The study suggests that both batch and continuous flow ultrasonic systems enhances enzymatic hydrolysis yield, reduces particle size of corn slurry and could be a potential effective pretreatment for corn slurry.*

Keywords. Corn slurry, ultrasonics, enzymatic hydrolysis, particle size, batch and continuous systems

Introduction

Currently, the United States is one of the biggest fuel ethanol producers in the world. The industry is rapidly growing; however, the relatively poor overall gains in energy require research to continually improve the technology. One aspect that warrants studies for improvement is the pretreatment process where huge amount of energy is used. Previous studies have shown that using ultrasonics is a potential pretreatment process that could enhance the production of fermentable sugars for fermentation (Khanal, et al. 2007; Montalbo-Lomboy, et al. 2008).

Two consequential effects of ultrasonication of corn slurry, e. g. cavitation and acoustic streaming, are considered as beneficial to the improvement of ethanol production. Ultrasound is defined as sound waves at a frequency above the upper range of the normal human hearing (>15-20 kHz). When ultrasound waves propagate through a liquid medium, these cause oscillations in pressure. The negative component of the ultrasonic pressures produces microbubbles though the phenomenon called cavitation (Suslick, 1988, Mason, 1999, Kardos and Luche, 2001). Because of surface tension, the presence of other bubbles, foreign bodies, and gradients in the pressure waves, each bubble becomes unstable beyond a critical size and eventually collapses violently. As the bubbles collapse, localized temperatures of up to 5000°K are achieved (Flint and Suslick, 1991). Ultrasound waves in liquid media also produce acoustic streaming, which facilitates the uniform distribution of ultrasound energy within the medium, convection of the liquid and dissipation of any heating that occurs (Faraday, 1831).

Ultrasonics has been widely used in various biological and chemical applications. Zhang et al. (2005) reported the use of ultrasonic treatment to enhance protein-starch separation for use in the wet-milling industry. Ultrasonics has also been employed to assist in the extraction of resveratrol from grapes (Cho et al., 2005). Li et al. (2004) utilized ultrasound treatment to enhance oil extraction from soybeans. Wood et al. (1997), studied ultrasonics to enhance ethanol yield from simultaneous saccharification and fermentation of mixed office paper. They achieved a 20% increase in ethanol yield from their sonicated samples.

Khanal et al. (2007) applied ultrasound to break down the particle size of milled commodity corn for subsequent improvement in sugar released in corn dry-milling. The authors reported a 3-fold increase in sugar production rate from the sonicated corn slurry. In this study, the authors will examine the effects of ultrasonic continuous system on the corn slurry in comparison with the ultrasonic batch system. The ultrasonic continuous system was initiated due to its scale-up potential for the ultrasonic technology in the ethanol industry. The ultrasonic continuous system involved the use of a "donut" shaped horn which vibrates in radial manner. These horns have been used widely in large scale operation for pretreating waste activated sludge for enhanced production of methane during anaerobic digestion (Khanal, Grewell, Sung and van Leeuwen, 2007). Additionally, this system is designed for heavy duty work which can withstand long operation hours. Based on these premises, the objective of the study was to compare the two ultrasonics systems (namely batch and continuous) in terms of enzymatic hydrolysis, relative energy gain and particle size reduction.

Materials and Methods

Materials

Dry ground corn was obtained from Lincolnway Energy LLC, Nevada, IA. The enzyme used was STARGEN^{TM} 001 (456 granular starch hydrolyzing units(GSHU)/g) from Genencor

International (Rochester, NY, USA), which contained *Aspergillus kawachi* α-amylase expressed in *Trichoderma reesei* and glucoamylase from *Aspergillus niger* that hydrolyzes starch dextrins into glucose.

Ultrasonic Continuous Systems

Corn slurry was prepared using 33% (m/v) dry ground corn in water obtained from Lincolnway Energy LLC, Nevada, IA. The ultrasonic continuous experiments were conducted using Branson 2000 series benchscale ultrasonic unit (Branson Ultrasonics, Danbury, CT) capable of operating at 3.3 kW and 20 kHz as shown in Figure 2. Corn slurry samples was pumped from a continuously stirred feed tank to an ultrasonic reactor where the Branson Ultrasonics 'donut" horn was placed (Figure 1). Volumetric flow rates were varied from 10 to 28 l/min at constant ultrasonic amplitude of $12 \mu m_{pp}$. After sonication, corn slurry samples (25ml) were collected then 10ml of 0.1M sodium acetate buffer (pH 4.5) and 18µl Stargen001 enzymes were added to it. The samples were then incubated for shorten enzymatic hydrolysis of 3 hours in a rotary shaker at 150 rpm and 32°C.

The authors would like to note that the total solid (TS) content of corn slurry after sonication was found to have reduced from 33% to 28% (m/v). The reduction is due to retention of some corn particles in the reactor, thus water flushing was done after every experimental run.

Ultrasonic Batch Systems

Ten milliliter (10ml) of 0.1M sodium acetate buffer (pH 4.5) was added to a 25ml corn slurry (28% m/v) sample. As it is noted earlier that the total solid content of samples after sonication in continuous systems decreased, thus the corn slurry concentration used for batch experiment was also reduced to maintain similar condition with the continuous system for the enzymatic hydrolysis.

The samples were then sonicated using Branson 2000 Series (Branson Ultrasonics, Danbury, Connecticut, USA) bench-scale ultrasonic unit for 20 and 40 s. The system is capable of operating at a maximum power output of 2.2 kW and a frequency of 20 kHz. The ultrasonic treatments were carried out in 50-ml polypropylene centrifuge tubes using three different amplitudes (power): low, medium and high (Table 1). The horn used was a standard 20-kHz half-wavelength catenoidal titanium with a flat 13-mm diameter face (gain = 1:8). Control indicates that sample was not treated with ultrasonication. STARGEN^{TM} 001 enzymes (18 µl) were added to the samples after sonication (including the control samples). The samples were then incubated for shorten enzymatic hydrolysis of 3 hours in a rotary shaker at 150 rpm and 32°C. Previous experiments (Montalbo-Lomboy, et al., 2008) indicated that sonicated samples reached the maximum hydrolysis faster than the control samples, thus a shorten enzymatic hydrolysis was used in this study. The control groups were samples that were not subjected to ultrasonics.

Table 1. Experimental conditions of ultrasonic batch systems

Parameters	**Power levels**		
	Low	**Medium**	**High**
Average power dissipated (J/s)	140-154	214-228	199-298
Amplitude (μm_{pp})	192	256	320

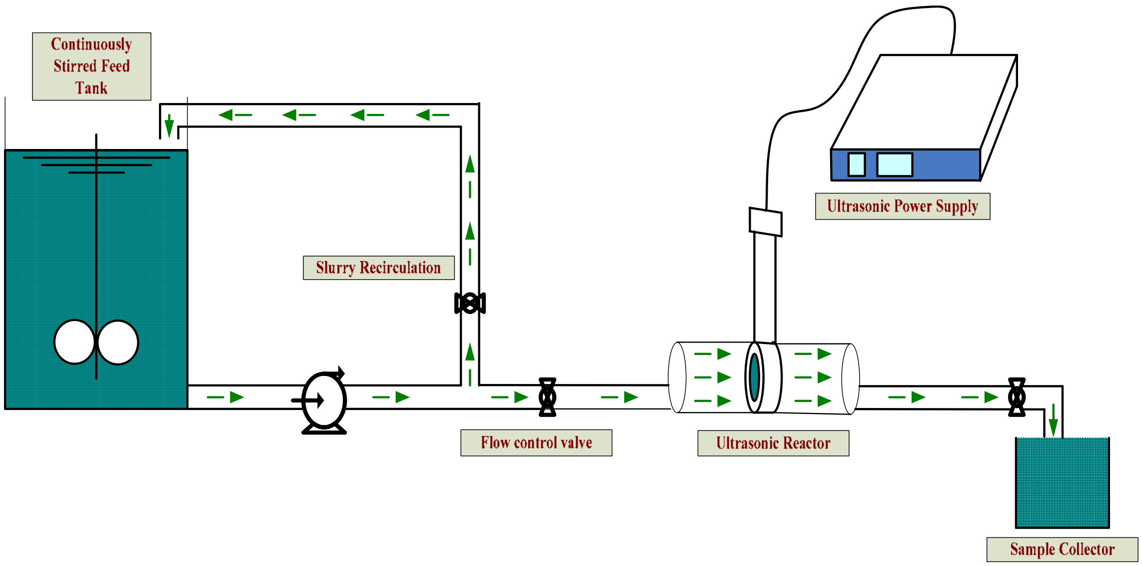

Figure 1. Ultrasonic continuous system experimental set-up

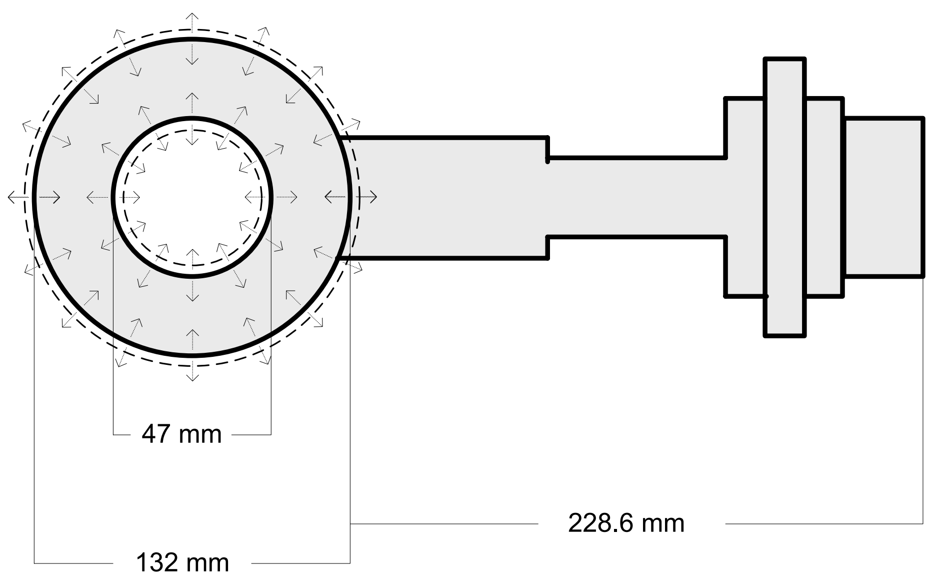

Figure 2. Branson ultrasonics "donut" shaped horn

Analytical Methods

After 3 h of enzymatic hydrolysis, 2 ml of 4M HCl-Tris buffer (pH 7) were added to the samples to stop the enzymatic reaction. The slurry was then centrifuged at 2,500 rpm for 15 minutes. Supernatant was then analyzed for reducing sugar concentration (glucose as standard) using a modified dinitrosalicylic acid (DNS) method as described in Khanal, et al. (2007). The particle size distribution of the corn slurry sonicated in batch and continuous systems were determined using Malvern particle size analyzer (Mastersizer 2000, Malvern Inc., Worcestershire, UK). Because 1200µm was the maximum particle size measured by the Malvern Mastersizer, the samples were screened through 1000µm screen prior to analysis. All experiments and analysis were conducted in duplicate and triplicate, respectively.

Ultrasonic Relative Energy Gain Calculation

The energy gain was established by comparing the total energy dissipated during sonication: Energy in (E_{in}) to the chemical energy of the additional sugar produced relative to the control group (Energy out, E_{out}). Assuming D-glucose as the standard monosaccharide used for reducing sugar analysis, and by further using the energy density of glucose (15,992 kJ/kg), the E_{out} is calculated according to Eq. 1. The total energy dissipated into the sample (E_{in}) in batch- and continuous-flow ultrasonic systems can be described by Eqs.2 and 3, respectively. The overall ultrasonic relative net energy gain can be calculated using Eq 4.

$$E_{out} = \left(RS_{sonicated} - RS_{control}\right) \times 15{,}740 \quad \text{[Eq. 1]}$$

$$E_{in} = \frac{P_{avg}\, t}{V} \quad \text{[Eq. 2]}$$

$$E_{in} = \frac{P_{avg}}{Vfr} \quad \text{[Eq. 3]}$$

$$Gain = \frac{E_{out} - E_{in}}{E_{in}} \quad \text{[Eq. 4]}$$

Where: *t* is sonication time (s)

V is volume (L)

Vfr is volumetric flow rate (L/s)

P_{avg} is average power (W)

RS is reducing sugar (g/L)

E_{in} and E_{out} is energy in and out (J/L)

Results and Discussion

Enzymatic Hydrolysis Yield

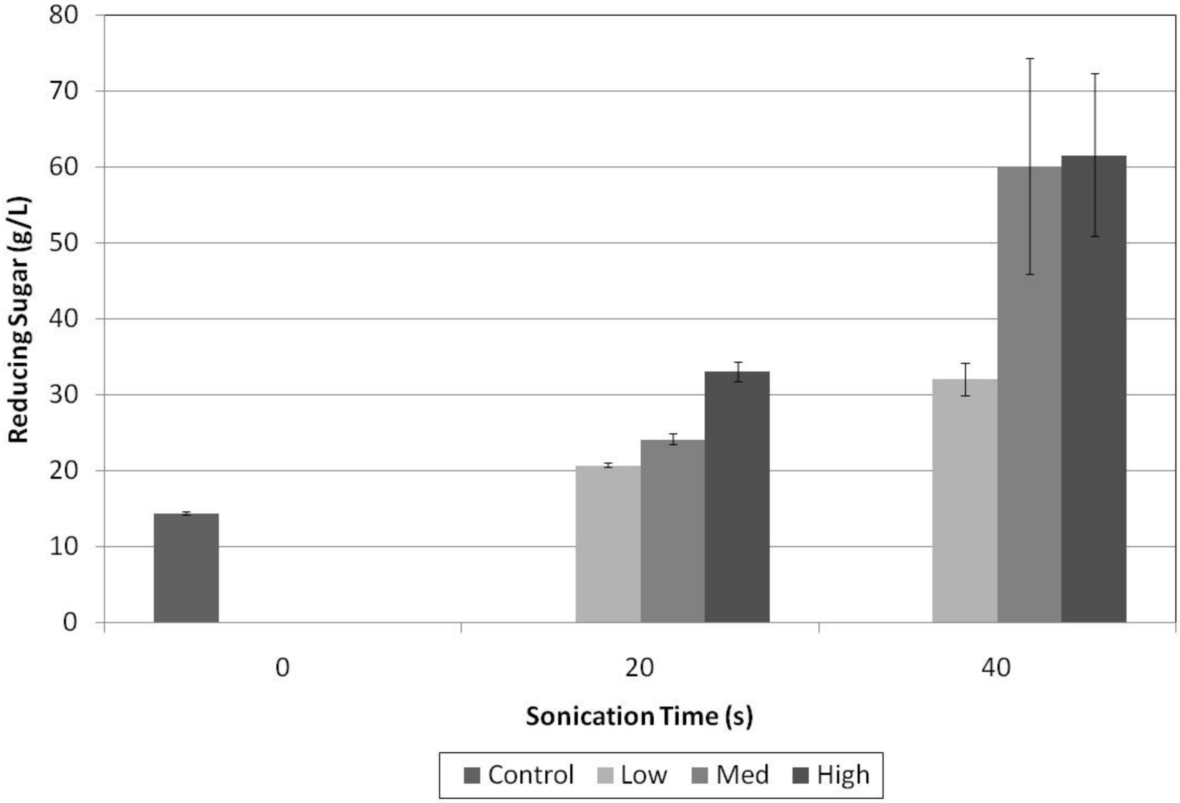

Figure 3. Reducing sugar yield of ultrasonic batch system

The reducing sugar yield of treated and untreated corn slurry sonicated in batch at various treatment conditions is shown in Figure 3. The sonication time at zero (0) corresponds to the control group where ultrasonication was not conducted. All the sonicated samples yielded higher reducing sugar than the control. It was seen that the sonicated samples have 0.4-3.3 times more sugar compared to the control. It was also seen that the additional sugar release was proportional to the ultrasonic power level (amplitude) and sonication time. Based on these observations, it was deduced that medium power setting at 40s treatment time gave the highest sugar yield over the conditions studied.

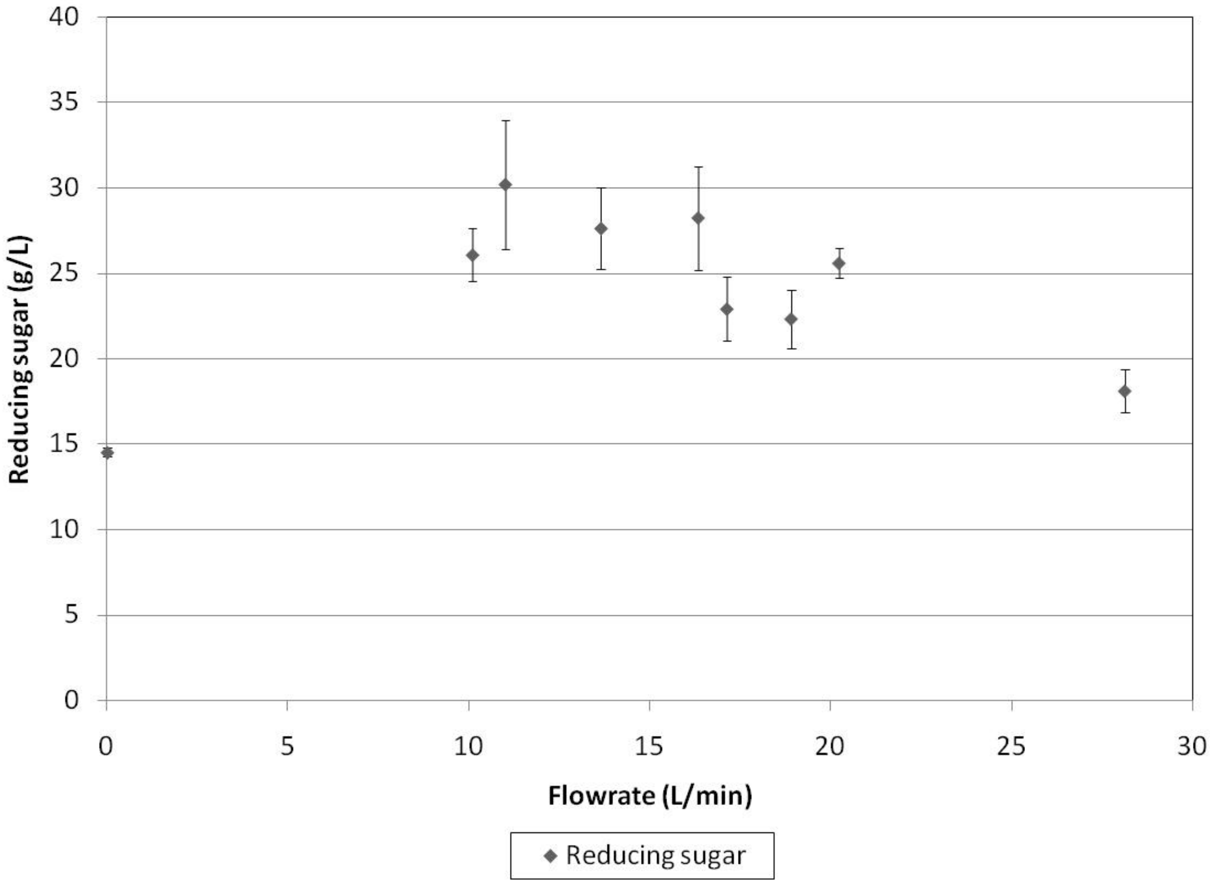

Figure 4. Reducing sugar yield of ultrasonic continuous system

Error! Reference source not found. shows the reducing sugar yield of treated corn slurry in a continuous flow ultrasonic system with a 3-h hydrolysis period. The volumetric flow rate was varied from 10 to 28 l/min. In this figure, a flow rate at 0 l/min corresponds to the control sample (untreated). The reducing sugar yield of the enzymatic hydrolysis ranged from 14-30 g/L. It is seen that the treated samples yielded about 24-100% more sugar compared to the untreated samples. Considering only the data presented, it could be assumed that as the flowrate increases, the reducing sugar yield decreases. However, it is observed that the difference in sugar yield between the sonicated and control in the continuous flow system is lower compared to the batch system. Further comparison between the two ultrasonic systems will be discussed in the later section.

Particle Size Analysis

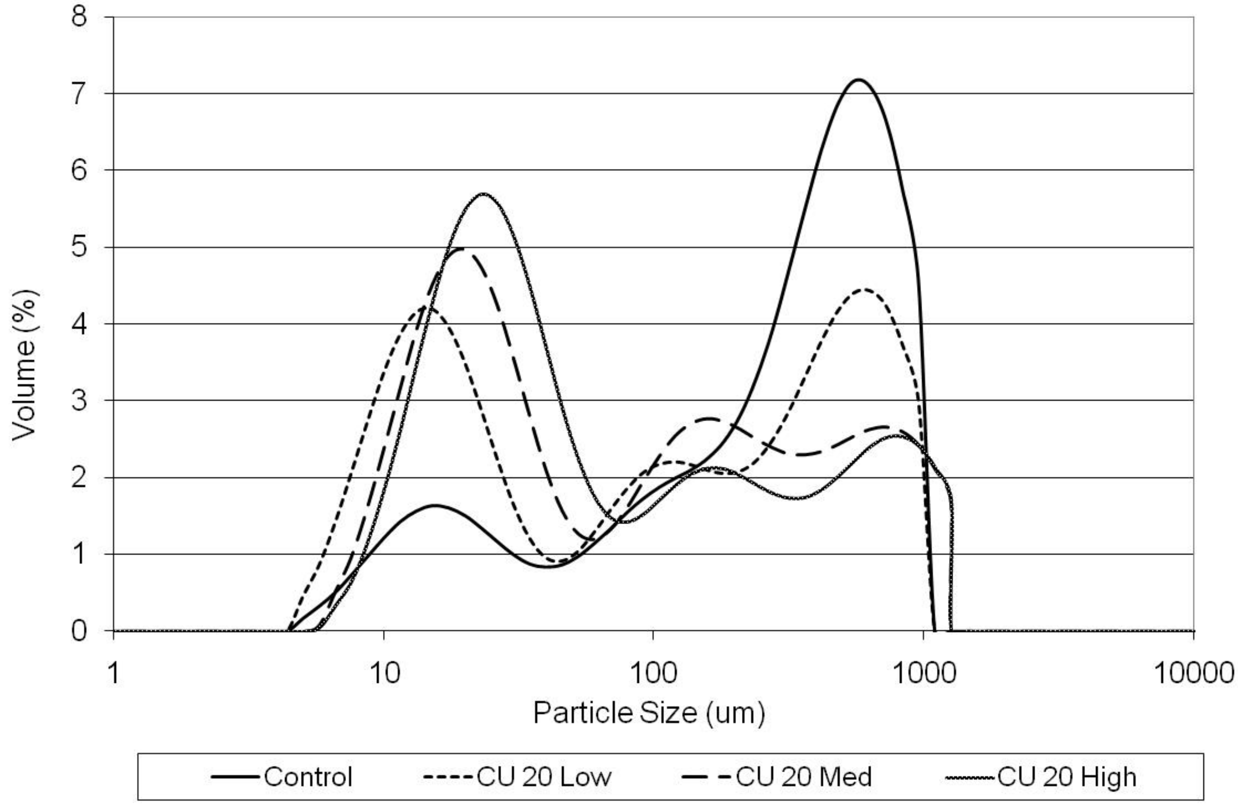

Figure 5. Particle size analysis of ultrasonic batch system at varying power input for 40s sonication time

In the past, a similar study was conducted on particle size analyses but was only limited to batch ultrasonic system and sonication experiments on lower total solid content (Khanal, et al., 2007). In this study, the particle size of the sonicated corn slurry in batch and continuous flow was

analyzed and compared. Figure 5,

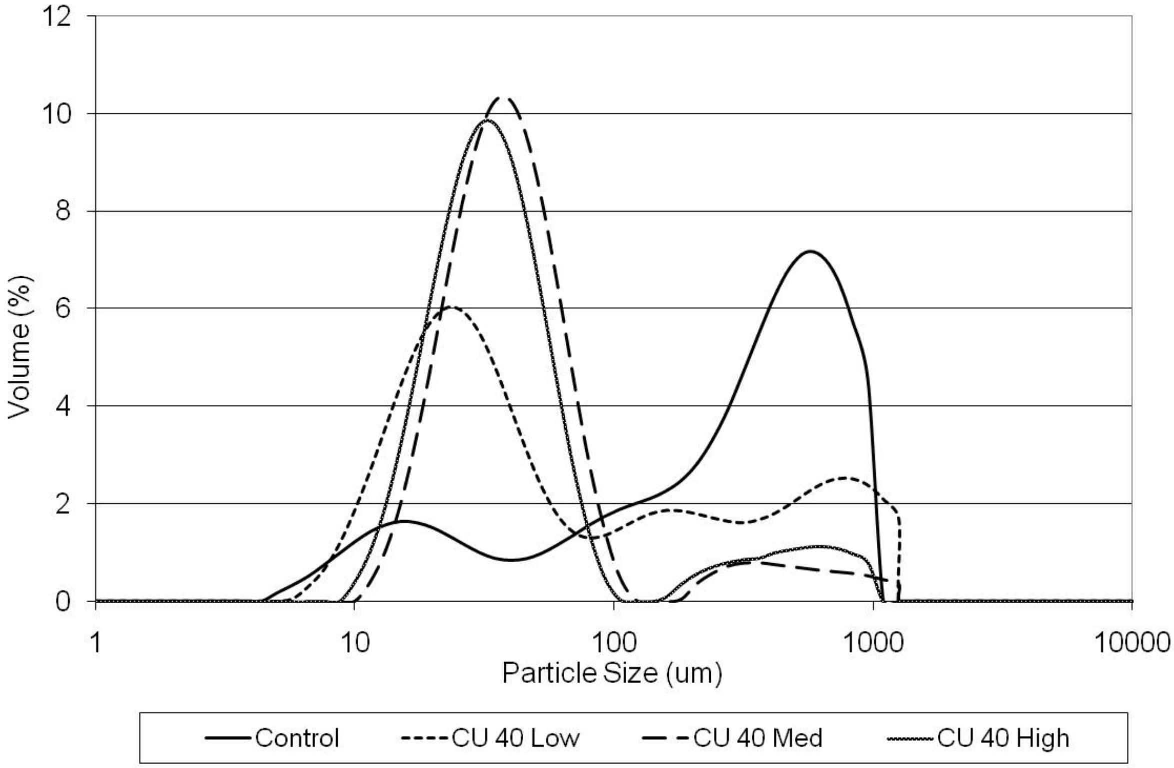

Figure 6, and Figure 7 shows the particle size analysis of corn slurry sonicated on batch at 20s, batch at 40s and continuous systems, respectively.

As seen, the particle size distribution has 2 inflection points in the control while in contrast there are 3 inflection points in the sample sonicated for 20 s. Interestingly, it is seen that with the 40 s treatment there is a single modal distribution. Additionally, the inflection point of the particle size distribution curve is shifted from 500 µm to approximately 20 µm following sonication. It was also seen that the relative volume within the inflection point increased as the ultrasonic power level increased, for both 20 s and 40 s of sonication. This data is in good agreement with Khanal et al., (2007), where particle size was found to be generally inversely proportional to ultrasonic treatment energy.

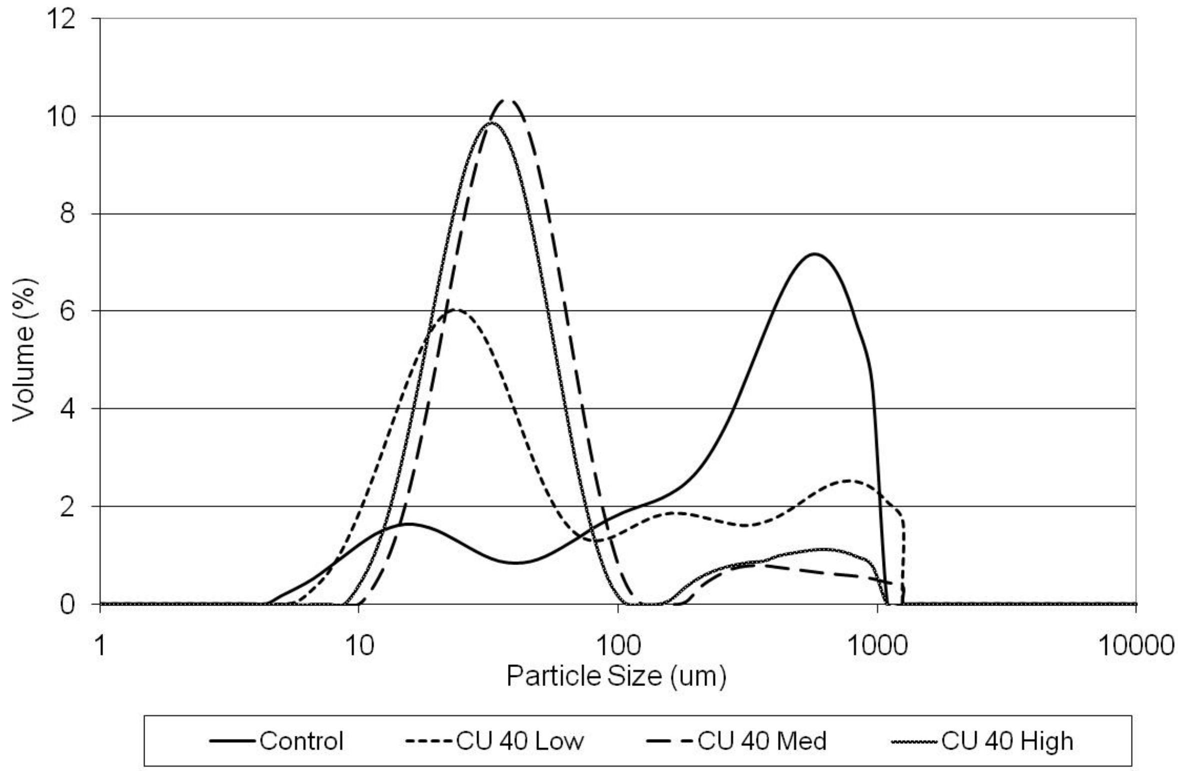

Figure 6. Particle size analysis of ultrasonic batch systems at varying power input for 20s sonication time

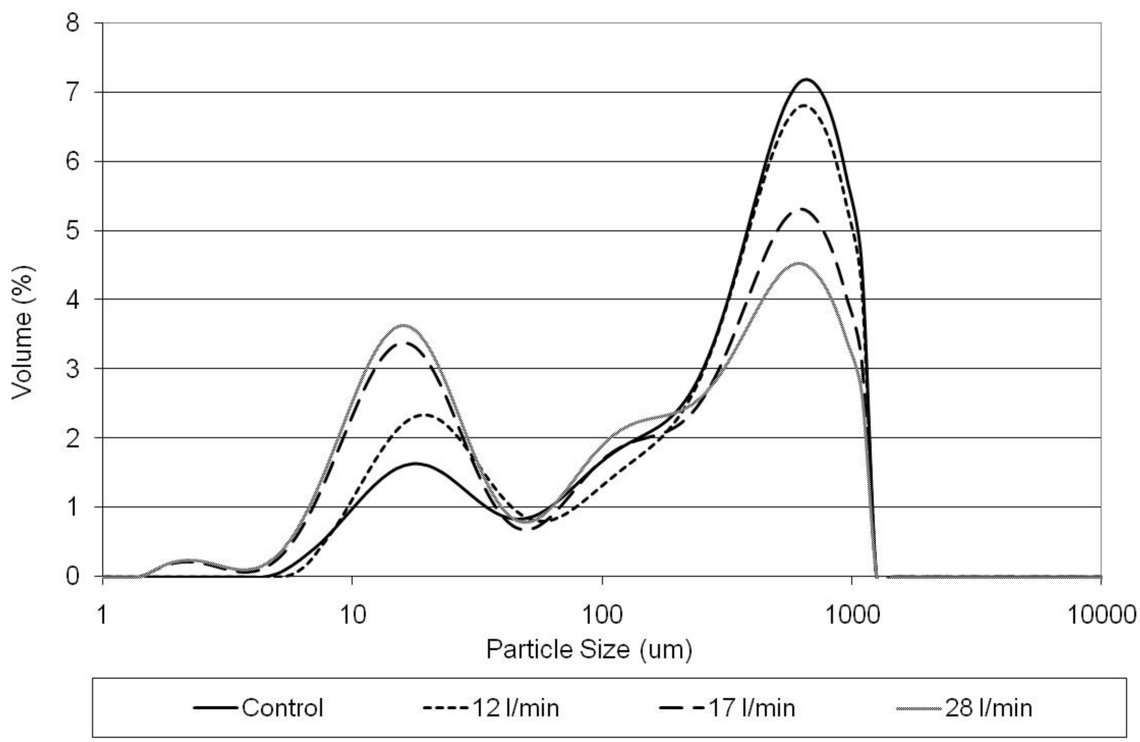

Figure 7. Particle size analysis of ultrasonic continuous systems at varying flowrate

Figure 7 shows the particle size distribution of the continuous flow experiment at various flow rates. Three flow rates were considered and compared with the control (unsonicated). It was observed that particle size reduction is proportional to the increasing flow rate of the continuous flow experiment. This trend could be due to the higher impact of corn particles on the donut horn at higher flow rates. However, at this point, it is still not clear why the lower flow rate condition, which gave lower particle size reduction, obtained a higher reducing sugar yield when it is expected to have longer ultrasound exposure. Further tests will be conducted in the future.

In Figure 8, the particle size distribution in the batch system is compared with particle size distribution for the continuous flow system. The highest particle size reduction was found at a flow rate of 28 L/min (**Error! Reference source not found.**), thus it was used in this plot for comparison. As expected, the ultrasonic batch system obtained higher particle size reduction than the ultrasonic continuous system. This is also observed in Figure 9.

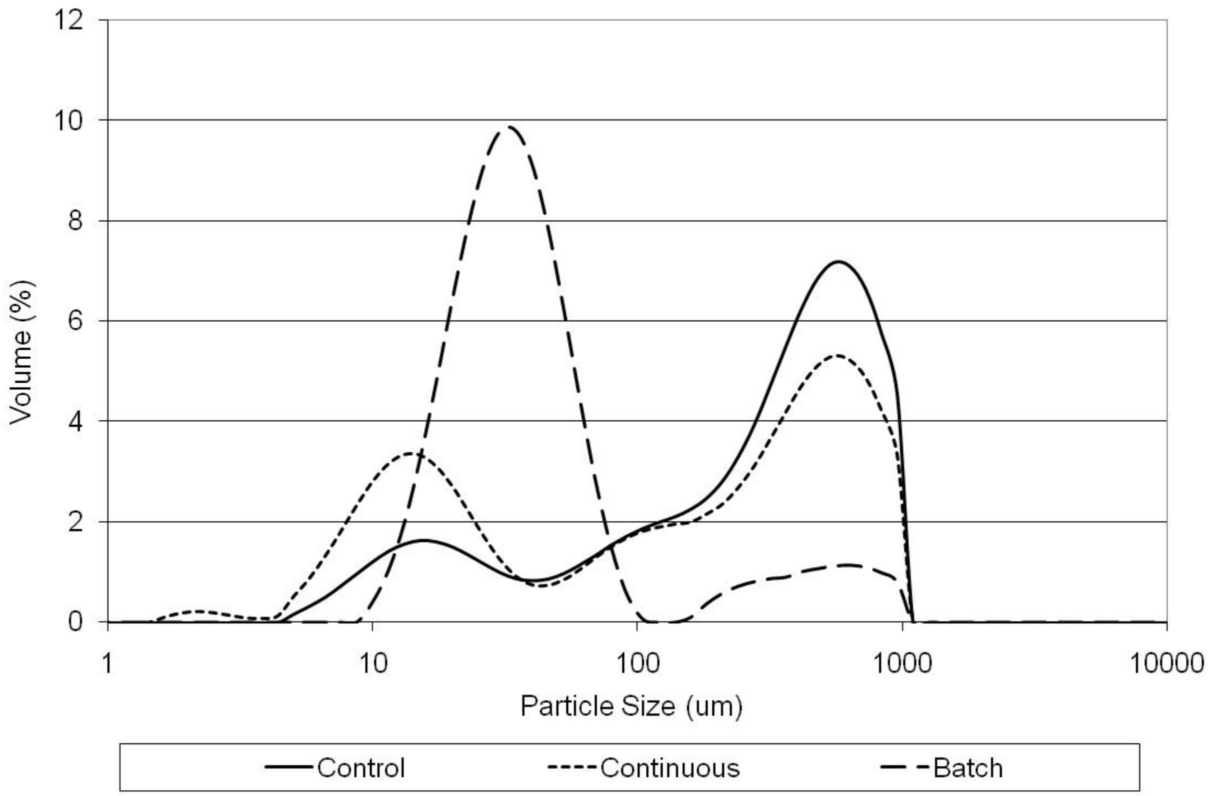

Figure 8. A comparison of the particle size analysis of ultrasonic batch and continuous systems

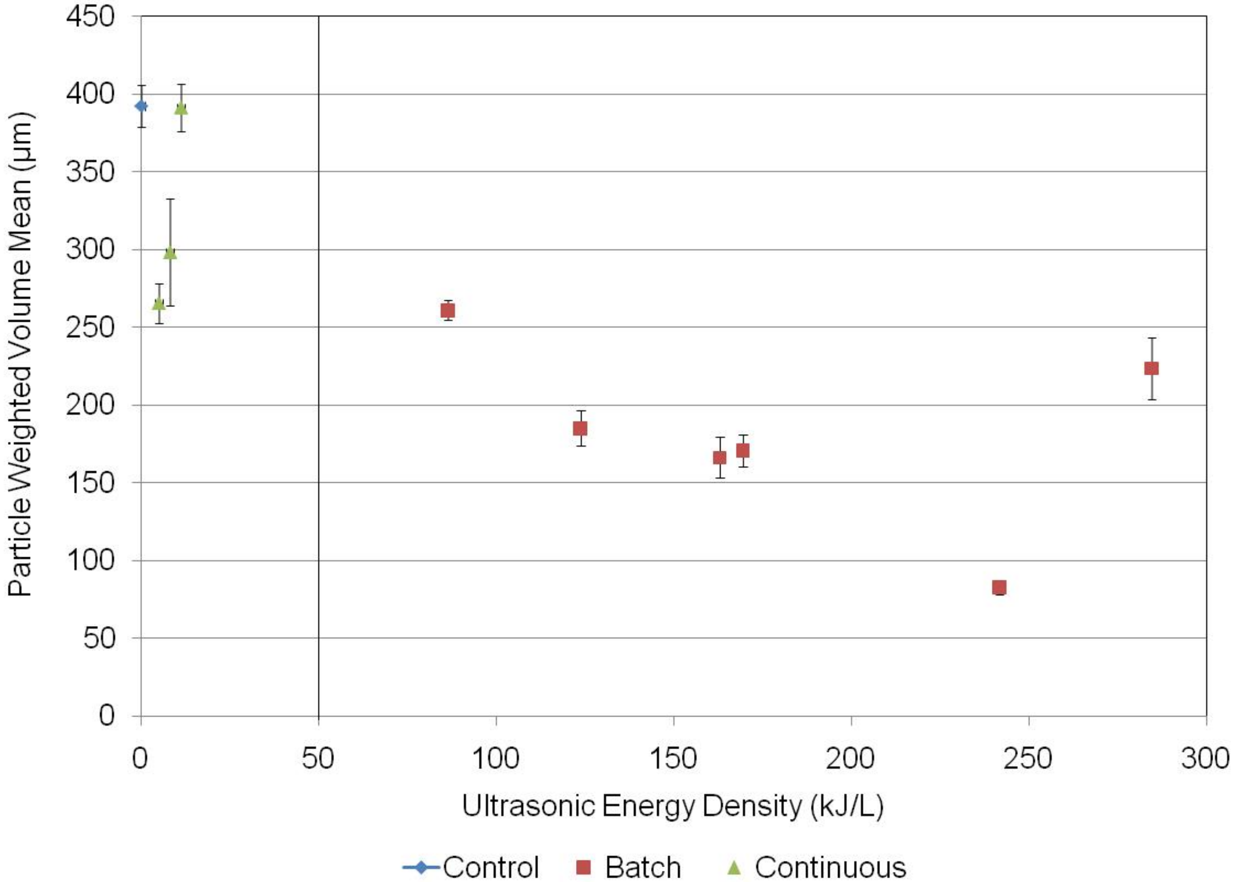

Figure 9. Effect of ultrasonic energy density on the particle weighted volume mean

Figure 9 shows the mean particle weighted volume mean (WVM) of corn slurry as a function of ultrasonic energy density. The ultrasonic energy density at 0 kJ/L indicates the untreated control sample. The division line in the figure separates the batch from the continuous ultrasonic system. The higher energy input in batch systems, resulted in more particle size reduction compared to the lower energy input required in continuous flow systems. It is seen that majority of WVM result is inversely proportional to the dissipated energy. This indicates that the correlation of particle size disintegration to the ultrasonic energy introduced will obtain similar trend whether it is a batch or continuous type of ultrasonic system.

To compare the two types of ultrasonic system studied; reducing sugar yield after enzymatic hydrolysis was illustrated as a function of ultrasonic energy density input, as shown in Figure 10. As seen in the figure, the continuous system's energy densities span a smaller range of 5-14 kJ/L while the batch system ranged from 87-224 kJ/L. As expected, the reducing sugar was proportional to the rise in ultrasonic energy density introduced in each corresponding system. It is seen that even though the batch system introduced more energy than the continuous system; there are some conditions where the reducing sugar obtained in batch is comparable to the continuous systems. This indicates that the increase in reducing sugar yield in the continuous system could also be due to other factors, e.g., pressure and flowrates; and does not only depend upon the ultrasonic energy input.

In Table 2, the ultrasonic relative energy gain for both batch and continuous system at varying condition were listed. All the treatment variations show a positive relative energy gain, indicating both systems to be energy efficient. It could be seen that if 1 Joule of ultrasonic energy is dissipated, the continuous systems released approximately 24 Joule – equivalent sugar released relative to the control, while the batch system released 2 Joule. Thus, it is evident that the continuous systems are more energy efficient than the batch system.

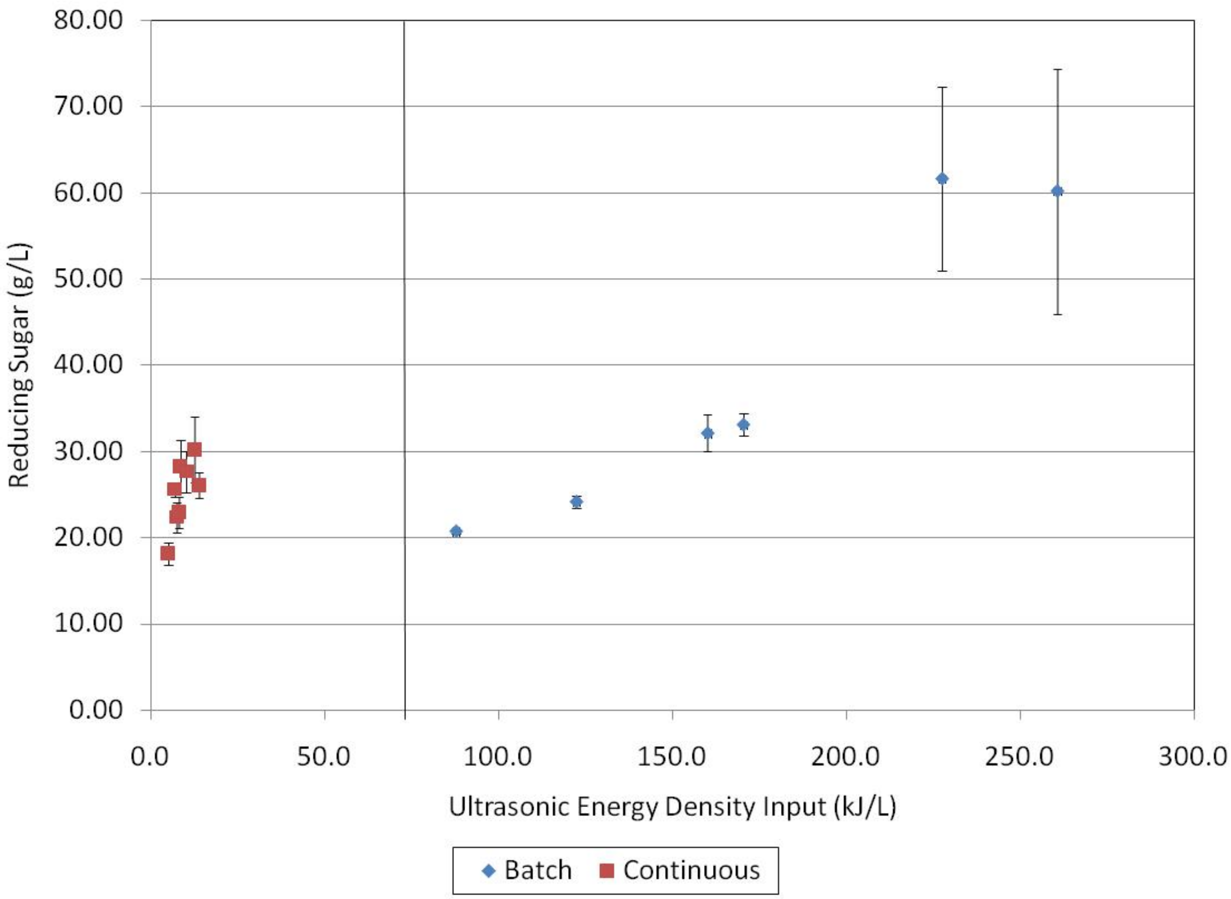

Figure 10. Reducing sugar yield of corn slurry as a function of ultrasonic energy density input

Ultrasonic Relative Energy Gain

Table 2. Ultrasonic relative energy gains of batch and continuous systems at varying conditions.

Batch		Continuous	
Ultrasonic conditions	**Relative Energy Gain (kJ/L: kJ/L)**	**Flowrate (L/min)**	**Relative Energy Gain (kJ/L: kJ/L)**
CU 20s – Low	0.14	28.13	10.68
CU 40s – Low	0.76	20.23	24.86
CU 20s - Med	0.26	18.91	16.06
CU 40s – Med	1.8	17.13	15.6
CU 20s – High	0.74	16.33	24.88
CU 40s - High	2.31	13.65	19.66
		11.00	18.92
		10.10	12.47

Conclusion

This study investigates the effects of ultrasonics on enzymatic hydrolysis of corn slurry in batch and continuous systems. The study established that higher reducing sugar yield is obtained in batch systems than in continuous systems. However, based on the ultrasonic energy density introduced and the ultrasonic relative energy gain, the continuous system is more efficient than the batch system. Both ultrasonic systems resulted in reduction in particle size of corn slurry. It was also found that the particle size reduction was proportional to the ultrasonic energy density input. This study concludes that batch ultrasonic treatment can be very effective in small-scale experiments. However, for large scale set-up, continuous system is recommended.

Acknowledgements

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