1 Effect of Frequency and Reaction Time in Focused Ultrasonic Pretreatment
2 of Energy Cane Bagasse for Bioethanol Production

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ABSTRACT

Pretreatment of lignocellulosic biomass is a critical steps in bioethanol production. Ultrasonic pretreatment significantly improves cellulose hydrolysis increasing sugar yields, but current system designs have limitations related to efficiency and scalability. This study evaluates the ultrasonic pretreatment of energy cane bagasse in a novel scalable configuration and by maximizing coupling of ultrasound energy to the material via active modulation of frequency. Pretreatment was conducted in 28% ammonia water mixture at a sample:ammonia:water ratio of 1:0.5:8. Process performance was investigated as a function of frequency (20, 20.5, 21 kHz), reactiontime (30, 45, 60 min), temperature, and power levels for multiple combinations of ammonia, water and sample mixture. Results indicated an increased enzymatic digestibility, with maximum glucose yield of 24.29 g / 100 g dry biomass. Theoretical ethanol yields obtained ranged from 6.47 to a maximum of 24.29 g/100 g dry biomass. Maximum energy attainable was 886.34 kJ/100 g dry biomass.

Keywords: Ultrasonic Pretreatment; Lignocellulose; Energy Cane; Enzymatic Hydrolysis; Ammonium Hydroxide

1. Introduction

Fuel ethanol derived from renewable resources has numerous advantages over conventional fossil fuels. Ethanol has a lower energy density than gasoline, but it also has a lower stoichiometric air-fuel ratio than gasoline, so a higher compression ratio can be achieved in the engine. These factors produce high output power and improved engine efficiency compared to gasoline-fueled vehicles. According to the Renewable Fuels Association, the US annual fuel ethanol capacity was 2.9×10^9 US gallons in 2002, an increase of 10^9 US gallons over the production level in 2000 ([Mosier et al., 2005](#)), showing that there was an expected demand for ethanol.

Bioethanol production from lignocellulosic biomass involves pretreatment, hydrolysis, fermentation and product separation or purification ([Mosier et al., 2005](#)). Pretreatment is necessary in order to overcome the recalcitrance of lignin and to reduce the overall cost for ethanol production because the cost of pretreatment is a significant factor affecting the ethanol selling price. However, 30% of the total processing cost in the biomass to ethanol conversion is provided by biomass pretreatment due to the required infrastructure and severe treatment conditions([Alvira et al., 2010](#)). There are a number of different pretreatments; depending on their general principle of operation they can be classified as chemical, biological and physical pretreatments ([Alvira et al., 2010](#)). One of the common chemical pretreatment of lignocellulosic biomass is treatment in alkaline conditions. The effect of ammonium hydroxide on pretreatment has been studied previously ([Behera et al., 2014](#); [Cao, 2012](#); [Chaturvedi & Verma, 2013](#); [Maurya et al., 2015](#); [Zhong et al., 2014](#)). Some newer examples include chemical pretreatment with ionic liquids, which is very effective in the pretreatment of energy cane, however the cost associated with this technique is high([Maurya et al., 2015](#); [Qiu et al., 2012](#)). Another effective method is a microwave-based ammonia pretreatment which was recently reported for sweet sorghum bagasse([Chen et al., 2012](#)). Biological pretreatment are also effective([Maitan-Alfenas](#)

[et al., 2015](#); [Maurya et al., 2015](#)), but generally require longer processing times(from days to weeks)with additionalsteps required to remove contaminants and yield high amounts of sugars.

More recently, ultrasound was considered as the practical pretreatment option as scanning electron microscopy images showed that ultrasound have the capacity to modify the surface structure of lignocellulosic biomass with beneficial effects on the saccharification process([Zhang et al., 2008](#)). Ultrasound technique is considered an emerging green technology that has the potential to reduce the reaction time and the chemical loading during pretreatment ([Bharadwaja et al., 2015](#); [Bussemaker & Zhang, 2013](#)). Ultrasound produces sonochemical and mechano-acoustic effects which affect the chemical and physical structures of lignocellulose when in a liquid medium via cavitation and heating ([Bussemaker & Zhang, 2013](#); [Yachmenev et al., 2009](#)). Cavitation occurs at low frequency (16 to 100 kHz) in which most of the ultrasound energy is dissipated into the medium whereas heating occurs at a higher frequency (> 100 kHz); in this case only small amount of energy is dissipated as heat. The common understanding of ultrasonic pretreatment is that the powerful vibrations caused by the ultrasonic waves severely damage the biomass structure which subsequently increase the yield of enzyme hydrolysis ([Mason et al., 1996](#)).

Various parameters affect the ultrasonic process performance such as frequency, power of operation, reaction time and temperature. The effect of ultrasonic frequency has been previously studied which showed that ultrasound frequency of operation plays an important role in the reaction. An optimum frequency was established for polymerization ([Kojima et al., 2001](#)) whereas other researchers concluded that the highest latex degradation occurred at the optimal time and frequency combination([Utara & Moonart, 2013](#)). However, these studies have different application and reactions from the ones investigated in this study. Even though the effect of ultrasonic frequency on disruption of organic biomass as a method of pre-treatment has been very recently shown to be relatively insignificant up to 50 kHz ([Bhasarkar et al., 2015](#)), current literature does not present the effect of frequency when using either a clamp-on transducer

(which allows for in-pipe continuous processing) or a multimode-variable-frequency load-tracking ultrasonic generator. In addition to the frequency, other pretreatment parameters that were shown to affect the performance are power, temperature and time, and were investigated herein. The ultrasonic performance is greatly influenced by the power level, since higher power produces greater cavitation that induces the structural change in the biomass ([Rehman et al., 2013](#)) but maximum power does not always lead to maximum yields. An increase in ultrasonic power leads to increase in the ultrasonic effects on liquefaction, dissolution times, and hydrolysis yields ([Sasmal et al., 2012](#)). Time-wise, it is reported that an ultrasonic treatment with alkali for short time period (5-10 min) did not achieve significant delignification compared to alkali pretreatment without ultrasound ([Sun & Tomkinson, 2002](#)) suggesting that reaction time needs to exceed these values in order for the pretreatment to have a significant effect in removal of lignin. Optimum temperature is required for the process reaction, which can be achieved by inserting the reactor in an oil bath to maintain the required temperature during sonication. In this study, parameters other than the frequency such as temperature and power were increased and the reaction time was varied to observe the effect of ultrasonic process performance to maximize the sugar production. This process was carried out based on the premise that intensification of parameters to high values will yield high sugars.

Energy cane, a sugarcane variety with high biomass yields, higher fiber and lower sugar content ([Kim & Day, 2011](#)) was used in this study. Chemical composition of energy cane was reported to be 43% cellulose, 24% hemicellulose, and 22% lignin. It contains 53.6% juice on wet basis, of which 9.8% are sugars (9.6% sucrose, 0.1% glucose and 0.1% fructose) ([Kim & Day, 2011](#)). Energy cane also has higher biomass yields per acre, with yields ranging from 25.5 to 44.2 tons (wet) per acre.

This study evaluated, for the first time to our knowledge, the unique combination of a novel ultrasonic system operated at various frequencies, with a timed study at high temperature for pretreatment of energy cane biomass. Different ratios of water and

ammonia mixtures (including very little water) were evaluated by perceiving the changes in chemical composition and enzymatic digestibility of untreated and treated lignocellulose. Virtually all ultrasound-processing studies described in literature are using ultrasonic baths and horn type transducers with their own transducer frequency. In this study a more specialized ultrasonic unit with a clamp-on transducer operating on the multimode, variable-frequency load-tracking principle was used to pretreat the lignocellulosic biomass with ammonium hydroxide at high temperature for different times with the evaluation the effects that time and water/ammonia concentration has on process performance and energy balance.

2. Materials and Methods

2.1 Substrate

Energy cane (variety L79-1002) was harvested at the Sugar Research Station of Louisiana State University Agricultural Center in Baton Rouge, LA (30.41° N, 91.17° W). The stalks were crushed to press - extract the juice in a roller mill (Farrel Corporation, Ansonia, CT) three consecutive times and the bagasse was stored in the freezer at -20°C. Before pretreatment, the energy cane was defrosted and boiled thrice with water for 6 hours at just below 100°C in a boiler to remove water soluble sugars. Then, the extracted biomass was sun dried to 7.64% moisture content dry basis and stored in sealed plastic bags. The drained water from the extraction process was collected for further sugar analysis. The bagasse particle size was less than 3mm.

2.2 Ultrasonic pretreatment

The pretreatment with ultrasound and ammonia as described further in this work was undertaken prior to enzymatic hydrolysis (i.e. the pre-treatment and the enzymatic hydrolysis were not undertaken simultaneously). Thus, the effect of the pretreatment can be decoupled from the effect that ultrasounds might have on enzyme configuration with respect to its folding conformation, as it has been recently shown that ultrasound affects the folding of protein

enzyme due to the microturbulence generated by ultrasounds and subsequent cavitation effects literature ([Bhasarkar et al., 2015](#)).

2.2.1 Effect of frequency

The ultrasonic pretreatment setup consisted of an ultrasonic generator (AMMM generators 400W, MPI Ultrasonics, Switzerland) connected to the clamp-on transducer (Branson 502/932R, 20 kHz, Mastersonic, Switzerland) fixed on a metal reactor tube of 250ml volume (Fig. 1). A torque of about 20 kg force was applied using a torque gauge on the ultrasonic converter clamping to the reactor tube according to the manufacturer specifications. Ultrasonic gel was applied between the clamp and the tubular reactor to enhance the transmission of the ultrasonic waves.

The generator was controlled and accessed by the National Instruments (NI-VISA Run-Time Engine 4.6.2), AMMM Labview (Lab View Run-Time Engine 2009 SP1) (National Instruments, Austin, TX, USA) based software for optimizing parameters including frequency and power. The transducer was driven by the generator in the amplitude-power mode and all other parameters were optimized accordingly. The impedance matching between the ultrasonic generator and transducers was automatically tracked by the system response via the built-in feedback system that adjusts the mode frequency of the process. Compared to the horn transducer, these clamp-on transducers can be scaled up easily and the tracking of load ensures maximum energy transfer to the material.

Three different sample combinations based on water and ammonia mixture with energy cane bagasse was loaded into a metal reactor tube individually and ultrasonic pretreatment was performed. The three different pretreatment combinations initially investigated were:

- (1) Energy cane (EC) bagasse with 28% NH_4OH and water at a ratio of 1:0.5:8 (weight basis)
- (2) EC bagasse soaked with 28% NH_4OH and water with the same ratio for 3 h and drained (performed in order to minimize the costs associated with the water processing).
- (3) EC bagasse with water at a ratio of 1:8.5 (no ammonia).

All three pretreatment combinations mentioned were exposed to three different frequencies (21 kHz, 20.5 kHz, and 20 kHz) for ultrasonic reacting time of 30 min with 100 W power. The solid samples were drained after the pretreatment and dried in an oven at 110 °C for 20 h. Untreated energy cane was used as control. All pretreatment experiments were performed in duplicates. The samples were then stored (at -20°C) in sealed plastic freezer bags until composition analysis and enzymatic hydrolysis was performed.

2.2.2 Effect of pretreatment time

The pretreatment was undertaken using the system as described above with a modification that allowed both the ultrasonic transducer and the reactor to be placed in an oil bath to achieve the higher process temperature (120-140°C) (Fig. 1). An equipment malfunction required the replacement of the 400 W ultrasonic generator with new, higher-performance generator with a maximum operating power of 1000 W with an updated AMMM Labview software. Thus, the non-soaked combination mixture samples were processed using the 400 W generator while the soaked sample mixtures were processed with 1000 W generator although a maximum power of 200 W was used for both the soaked and non-soaked samples.

The same combination of biomass, water and ammonia as described above were used in this experimental setup, with the additional of a fourth combination (namely the bagasse + water soaked for 3 h followed by draining). The samples were loaded into a metal reactor tube, and placed in heated oil bath. Prior to inserting the reactor tube, the mineral oil in the bath was heated to a steady state temperature between 120 and 140 °C by a Polystat immersion circulator equipped with a temperature controller (Cole Parmer LLC, Vernon Hills, IL). The temperatures of oil bath and the sample were measured by K-type thermocouple sensors and were recorded using a Pico data logger (Pico Technology, Tyler, TX, USA). The ultrasonic pretreatment began when the sample mixture inside the reactor reached the steady state temperature (120 to 140 °C). Each of the four different combination mixtures mentioned were

exposed to an ultrasonic frequency of 20 kHz at 200 W for three different pretreatment times (30, 45, and 60 min).

2.3 Chemical composition of energy cane

Untreated and ultrasonically pretreated biomass samples were analyzed for their composition in terms of glucan (cellulose), xylan (hemicellulose), lignin, extractives, solids and ash using the National Renewable Energy Laboratory (NREL's) Laboratory Analytical Procedures (LAPs #42618, 42619, 42620, 42621, and 42622), which are incorporated here via reference ([NREL, 2015](#)). All the samples were processed in duplicates. National Institute of Standards and Technology (NIST) energy cane standard was analyzed simultaneously with the untreated and pretreated samples to ensure the accuracy of the procedures. The results were analyzed according to the corresponding LAP using high performance liquid chromatography (HPLC) (Agilent 1200 Series company, Santa Clara, CA). The percentage of glucan loss, xylan loss and lignin removed were calculated using the following equations adapted from ([Qiu et al., 2012](#)).

$$\% \text{ Glucan Loss} = 1 - \frac{\% \text{ glucan in treated biomass} * \% \text{ recovered solids}}{\% \text{ glucan in untreated biomass}} \quad (1)$$

$$\% \text{ Xylan Loss} = 1 - \frac{\% \text{ xylan in treated biomass} * \% \text{ recovered solids}}{\% \text{ xylan in untreated biomass}} \quad (2)$$

$$\% \text{ Lignin Removed} = 1 - \frac{\% \text{ lignin in untreated biomass} * \% \text{ recovered solids}}{\% \text{ lignin in untreated biomass}} \quad (3)$$

2.4 Enzymatic hydrolysis

The hydrolysis of untreated and pretreated energy cane was performed using a combination of two commercially available enzymes, Spezyme CP (cellulases) (Genencor, Danisco US Inc., Rochester, NY) and Novozyme 188 (cellobiases) (Sigma Aldrich, St. Louis, MO). A total weight of 50 g (dry weight) of treated and untreated energy cane samples were prepared for enzymatic hydrolysis using a slightly modified NREL's LAP TP-510-43629 procedure([NREL, 2015](#)). The activity of the enzymes used is significant and was evaluated using NREL's LAP TP-510-42628. The substrates were hydrolyzed simultaneously with 30

FPU/g glucan of Spezyme CP and 30 CBU/g glucan of Novozyme 188, which are half the recommended values in order to evaluate potential cost reductions when using lower enzyme loading. Hydrolysis was performed as ascribed by LAP-510-43629 using a pH 4.8 sodium citrate buffer, at a temperature of 50°C ± 1°C. When the immersion oil bath was used to evaluate the effect of temperature, the enzyme concentration for hydrolysis after pretreatment was that recommended by TP-510-43629 (60 FPU/g glucan of Spezyme CP and 60 CBU/g glucan of Novozyme 188). Experiments were run in duplicates. Hydrolyzed liquid samples were collected after 24, 48, and 72 h. After hydrolysis, the percentage of glucose released from the samples were determined and analyzed by HPLC with a BioRad Aminex HPX-87P (P), lead form, 300 mm x 7.8 mm (ID), 9 µm column and a differential refractive index detector (G1362A Agilent, Santa Clara, CA).

2.5 Analysis of hydrolyzed energy cane

The samples collected after 24, 48 and 72 h of enzymatic hydrolysis were heated on a hot plate at above 100 °C in order to deactivate the enzymes present. The samples were then filtered and collected in HPLC vials for sugar analysis. The theoretical percentage of cellulose digestibility was calculated using the formula provided in NREL's LAP TP-510-43630 as described below:

$$\% \text{ Glucose Yield} = \frac{[\text{Glucose}^*] + 1.053 [\text{Cellobiose}]}{1.111 f [\text{Biomass}]} \quad (4)$$

Where, Glucose* is the glucose concentration (g/L), Cellobiose is the cellobiose concentration (g/L), biomass is the initial dry biomass concentration before the enzymatic hydrolysis (g/L), 1.053 is the multiplication factor to convert cellobiose into equivalent glucose, f is the cellulose fraction in dry biomass (g/g) and 1.111 is the factor to convert cellulose into equivalent glucose. Ethanol yields were reported based on theoretical fermentation yield from the available released sugars, including those sugars extracted prior to pretreatment in the hot-water washing steps (but without the sugars extracted by pressing the juice out of the stalks).

3. Results and Discussion

3.1 Effect of frequency on composition

The composition data of the untreated and pretreated energy cane bagasse were analyzed and compared based on dry weight (Table 1). The composition of untreated energy cane bagasse was 39% glucan, 19.3% xylan and 25.3% lignin which were comparable to composition analysis of energy cane bagasse reported in literature ([Aita et al., 2011](#)). Total lignin removal was low after the pretreatment. As indicated by the high solid recovered percentage of the pretreated samples, there was no significant removal of glucan and xylan. No statistically significant variation in composition in terms of glucan yield ($\alpha = 0.05$) was observed with changes in frequency([Methrath Liyakathali, 2014](#)).Also there is no reduction in the lignin % for the different frequencies and different combination mixtures. These results lead to the conclusion that the low pretreatment processing temperatures (80 to 90 °C) may have prevented lignin removal, as lignin is reported to dissolve between 140 and 160 °C even in the presence of ammonia([Puri & Pearce, 1986](#)). However, 18.15% higher glucose yield could be obtained for the pretreated sample compared to the untreated control.

3.2 Effect of frequency on enzymatic hydrolysis

As the composition analysis does not necessarily indicate the results in terms of sugars available for fermentation, an enzymatic hydrolysis of untreated and pretreated energy cane bagasse was performed. The cellulose digestibility obtained for the pretreated energy cane combination mixtures at an enzyme loading of 30 FPU Spezyme CP and 30 CBU Novozyme 188/g glucan was slightly higher compared to untreated energy cane (11.14%, 15.91% and 15.92%) after 24, 48 and 72 h of hydrolysis, respectively. There was no significant variation among the various pretreated samples processed at the different frequencies ($\alpha = 0.05$)(Fig. 2) ([Methrath Liyakathali, 2014](#)).These results are in agreement with data reported in literature([Bhasarkar et al., 2015](#)) for the effect of various frequencies on ultrasonic enzymatic

fuel desulfurization. That study used numerical modeling of cavitation bubble dynamics to conclude that the effect of ultrasound frequency is more pronounced only at values above 50 kHz (negligible for the range of 20 – 50 kHz). However, that study used ultrasound simultaneously with the enzymatic process, thus a direct comparison with the current results cannot be made.

The results indicate that there was no significant removal of lignin from the energy cane bagasse after the pretreatment at the different frequencies, even in the presence of ammonia. The increase in glucose digestibility compared to the non-pretreated sample can be inferred from the increased % glucan observed in Table 1. Higher glucan digestibility for pretreated samples was obtained which can be attributed to the effect of ultrasonic treatment. Ultrasound waves generates micro-turbulence within the system causing enhanced mass transfer effect ([Singh et al., 2014](#)). Moreover, ultrasound induces liquid cavitation; the bubbles thus formed grow and ultimately collapses causing massive shock waves. These shock waves cause the biomass particles to collide which results in rupturing the lignin matrix by releasing large amount of energy ([Doicrycz & Suslick, 1990](#); [Singh et al., 2014](#)).

3.3 Effect of pretreatment time at high temperature on composition

The process temperature was recorded in real time and the graphs were plotted using the data obtained for different combination mixtures samples at various reaction times (Fig. 3). The average reaction temperature was 120 to 140 °C for all the combination mixtures. A gradual temperature evolution was obtained. The chemical composition of the pretreated energy cane combinations is summarized in Table 2. Ultrasonically pretreated samples at temperatures above 120°C, 20 kHz, 200 W and 30, 45, 60 min reaction time showed an increase in glucan compared to samples processed in the temperature range of 80 to 90°C as noted in Table 1.

Non-soaked mixtures at 200 W

The % glucan obtained for the energy cane with ammonia and water at 30, 45 and 60 minutes reaction time were 43.77%, 45.44% and 50.93% respectively. There was a 21.5%

1 increase in the glucan obtained for the energy cane with ammonia and water treated for 60
2 minutes reaction time compared to the untreated energy cane control. Lower reaction time
3 resulted in higher glucan yield. The amounts of recovered solids (in%) were low for the mixture
4 controls treated without ultrasonic (0 kHz) compared to the ultrasonically pretreated mixtures
5 (20 kHz). There was a significant difference ($\alpha = 0.05$) between the glucan yield for the mixtures
6 treated with and without ultrasonication, in both the energy cane with ammonia and energy cane
7 without ammonia combination mixtures (Table 2).

8 The percent lignin removal, glucan and xylan loss for all the non-soaked combination
9 mixtures are summarized in Table 3. The lignin removal was calculated based on the %-
10 recovered solids after the pretreatment. The glucan losses were higher for untreated mixture
11 controls (0 kHz) compared to the pretreated mixtures (20 kHz). These values indicate that even
12 if the lignin for the pretreated samples was not removed completely from the substrate after
13 pretreatment, they were depolymerized enough to increase for enzyme conversion
14 performance. This effect might be due to incomplete removal of acid insoluble lignin, since after
15 15 minutes of ultra-sonication, other researchers reported an increase in the acid insoluble
16 lignin, followed by a gradual decrease of lignin([García et al., 2012](#); [Singh et al., 2014](#)). Other
17 studies also observed similar results where higher lignin content was observed at longer
18 reaction times, attributed to repolymerization of lignin by the free radicals generated during
19 cavitation ([Singh et al., 2014](#)).

20 *Soaked and drained mixtures at 200 W power*

21 The glucan yields for the mixture with ammonia at 60 min was 47.65%, which was
22 greater than the 38.12% of the mixture without ammonia. The glucan yields obtained for the
23 energy cane, with only water at 30, 45 and 60 minutes reaction times were 30.81%, 33.07% and
24 33.22% respectively, These values were low compared to the values of 42.90%, 42.40% and
25 40.56% respectively, obtained for the energy cane with ammonia and water at the same times

(Table 4) indicating the beneficial effect of ammonia in the mixture with respect to process performance([Behera et al., 2014](#); [Chaturvedi & Verma, 2013](#)).

It was observed that the soaked energy cane mixture combinations showed increased lignin removal compared to the non-soaked combinations (Fig.3), which is also advantageous in terms of water utilization. Soaking of biomass in water for 3 h causes swelling of biomass which results in reduced crystallinity and increased surface area, which enhances the structural disruption of biomass. The lignin-carbohydrate bond is also weakened and results in reduced lignin percent for pre-soaked samples ([Suresh et al., 2014](#)). The glucan loss was higher not only for the mixtures without ammonia compared to mixtures with ammonia, but also for the untreated control (0 kHz) compared to the treated mixtures (20 kHz) mixture. Statistical variations were significant for glucan and lignin loss between all the combination mixtures and among all the different reaction times for the same combination mixture([Methrath Liyakathali, 2014](#)).

There was a significant weight loss in the samples pretreated at different reaction times compared to the samples pretreated at different frequencies. This showed an increase in the percent lignin removed (Table 3).

3.4Effect of reaction time on enzymatic hydrolysis

Cellulose digestibility results for all the four combination mixtures (non-soaked and soaked& drained) of ultrasonically pretreated energy cane are summarized in Fig.4. A maximum cellulose digestibility of >44.05% was achieved after 72 hours for samples pretreated for 60 min in both cases of non-soaked and soaked/drained combinations. In comparison, the controls showed a digestibility of 19.04%, 19.37% and 20.44% at 24 h, 48 h and 72 h of hydrolysis respectively. There was a relatively linear increase in the cellulose digestibility based on hydrolysis time (24, 48 and 72 hours) as well as the reaction time (30, 45 and 60 min)for each combination samples.

1 A cellulose digestibility of 33.88% was observed after 72 hours for non-soaked samples
2 treated with water and ammonia mixture for 30 minutes followed by 43.62% and 44.05% for 45
3 60 min respectively. Similarly for non-soaked samples treated with water only, cellulose
4 digestibility 17.93%, 21.01% and 22.31% was obtained after 30, 45 and 60 min respectively. For
5 pre-soaked samples treated with water and ammonia mixture, cellulose digestibility of 21.88%,
6 38.95% and 43.73% was obtained for reaction time of 30, 45 and, 60 min. For pre-soaked
7 samples treated with water alone, cellulose digestibility of 20.51%, 20.51% and 20.53% was
8 obtained for reaction times of 30, 45 and 60 min.

9 The increased cellulose digestibility at longer reaction time is due to the longer exposure
10 to the microjetting and microstreaming mechanisms that affect the biomass structure ([Esfahani &](#)
11 [Azin, 2012](#); [Peregrine, 1994](#); [Suresh et al., 2014](#)). These effects increase the enzyme/substrate
12 affinity by increasing the mass transfer rate of enzyme and substrate within the system as well
13 as the rate of conversion of reactants to sugars ([Singh et al., 2015](#)). The cellulose digestibility of
14 the untreated controls for almost all mixture combinations were low compared to the pretreated
15 samples (except for the samples soaked in plain water) (Table 3). The cellulose digestibility of
16 the soaked samples were higher than non-soaked sample among the controls only, as expected
17 since the biomass was exposed to the chemicals for longer times ([Aita et al., 2011](#)). Therefore,
18 the results for ultrasonically pretreated samples vary with different combinations and
19 pretreatment time, and increasing pretreatment temperature improved the efficiency of
20 pretreatment and enzymatic digestibility of pretreated samples.

21 In this study, the pretreatment temperature of all the samples were between 120 and
22 140 °C, and a maximum pretreatment time of 60 minutes resulted in significant improvements of
23 the process efficiency. As per the enzymatic hydrolysis data, energy cane with ammonia and
24 water, both soaked and non-soaked samples yielded a maximum of cellulose digestibility (Table
25 3). The cellulose digestibility (after 72 hours) for the pretreated energy cane with water and
26 ammonia for different reaction time showed significant variations.

3.5 Energy Balance

Energy balance for soaked and non-soaked samples was calculated using the theoretical ethanol yield obtained not only from the hydrolysis but also from the small amounts sugars released in the initial hot-water washing step, as this sugar would also be available for fermentation in a commercial operation. Based on the fact that soaked samples utilized less water, the overall energy consumption for the pretreatment was expected to reduced as heating water utilizes significant amount of energy(water drained from the samples after 3 hours = grams of water saved).Therefore, energy saved compared to non-soaked samples for heating can be calculated via a simple energy balance (eq. 4)

$$Q = M C_p \Delta T \quad (4)$$

Where, $C_p = 4180 \text{ J / kg K}$ is the specific heat of water

ΔT is the difference in temperature = $138.69 - 25 = 113.69^\circ\text{C} = 113.69 \text{ K}$

M = Mass of water drained in kg

$Q = 0.06 \text{ kg} * 4.18 \text{ kJ / kg K} * 113.69 \text{ K}$

$Q = 28.51 \text{ kJ}$ of energy required to heat water.

The highest energy (B:A:W soaked) produced was 886.34 kJ per 100 g dry biomass. The energy produced for each pretreated combination mixtures of non-soaked and soaked samples are summarized in Table 6. The optimum sonication power of ultrasound should be in range of 2-10 W/cm³ ([Yachmenev et al., 2009](#)); in the present study, it was only 0.8 W/cm³ which partially explains the lower yields. Higher sonication power also yields more glucan, since the formation of the bubbles at the tip of the transducer is conducive to better transfer of energy into the medium ([Gogate et al., 2011](#)).

The energy and cost of ultrasonic pretreatment in a larger scale needs to be investigated in the future. The energy required for ultrasonic pretreatment noted in literature utilized less energy compared to other techniques($7.2 \times 10^4 \text{ J/g}$ for ultrasound compared to steam explosion

and autoclave which were 9.9×10^4 J/g and 23.3×10^4 J/g respectively)([Velmurugan & Muthukumar, 2012](#)).

4. Conclusion

The frequency for various pretreated samples had no significant effect on glucan loss for the frequency range investigated. No differences were observed between different combination mixtures for a particular frequency based on compositional analysis and cellulose digestibility. However, parameters such as time, temperature and power level enhanced the ultrasonic pretreatment process. Lignin removal for energy cane bagasse with NH_4OH and water non-soaked and soaked samples were 24% and 34% respectively for 60 minutes reaction time, which exhibited significant % cellulose digestibility of almost 44% for both the samples compared to untreated. SEM images revealed changes in biomass structure post pretreatment.

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Figures

Figure 1. Process set-up for ultrasonic pretreatment of biomass

Figure 2. Percent (%) Cellulose digestibility after enzymatic hydrolysis at different ultrasonic frequencies

Figure 3. Pretreatment processing temperature evolution of the biomass and the oil medium for Biomass: Ammonia: Water combination

Figure 4. Cellulose digestibility after enzymatic hydrolysis of energy cane pretreated with (a) ammonium hydroxide and water (b) water only (c) ammonium hydroxide and water soaked for 3 hours before pretreatment (d) water soaked for 3 hours before pretreatment

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(*) Oil bath for studying the effect of reaction time only

Fig.1. Process set-up for ultrasonic pretreatment of biomass

EC – Energy cane bagasse control

B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts

B:W – Biomass : Water in 1:8.5 parts

* Modified procedure data

Fig.2. Percent (%) Cellulose digestibility after enzymatic hydrolysis at different ultrasonic frequencies

Temperature Evolution during Pretreatment

Fig.3. Pretreatment processing temperature evolution of the biomass and the oil medium for Biomass: Ammonia: Water combination

Fig.4. Cellulose digestibility after enzymatic hydrolysis of energy cane pretreated with (a) ammonium hydroxide and water (b) water only (c) ammonium hydroxide and water soaked for 3 hours before pretreatment (d) water soaked for 3 hours before pretreatment

Table 1
Composition Analysis of treated and untreated energy cane bagasse

Pretreatment Conditions				Biomass component* (% dry weight)				Recovered solids**
Mixtures	Time (min)	Power (Watt)	Frequency (KHz)	Lignin	Glucan	Xylan	Arabinan	
B:A:W	30	100	21	30.85 ± 0.35	43.11 ± 0.30	28.71 ± 0.62	2.05 ± 0.09	97.81 ± 0.33
			20.5	32.02 ± 0.64	42.40 ± 0.20	26.91 ± 0.14	1.33 ± 0.08	97.07 ± 0.35
			20	30.98 ± 0.62	42.39 ± 1.21	28.86 ± 0.54	1.80 ± 0.27	98.52 ± 1.28
B:W	30	100	21	30.67 ± 0.46	43.72 ± 0.64	27.76 ± 0.47	1.90 ± 0.21	99.79 ± 0.57
			20.5	29.98 ± 0.19	44.04 ± 0.20	26.33 ± 2.04	1.69 ± 0.21	100 ± 0.67
			20	32.03 ± 0.68	46.08 ± 0.11	28.65 ± 0.34	1.88 ± 0.16	98.64 ± 1.10
B:A:W 3Hrs soaked	30	100	21	26.04 ± 0.55	43.26 ± 0.88	28.55 ± 1.39	2.36 ± 0.66	99.62 ± 1.15
			20.5	29.68 ± 0.91	44.44 ± 1.11	26.27 ± 1.32	2.34 ± 0.64	98.92 ± 0.72
			20	29.68 ± 0.91	44.44 ± 1.11	26.27 ± 1.32	2.34 ± 0.64	98.92 ± 0.72
Untreated energy cane				25.3 ± 0.62	39.00 ± 0.87	19.3 ± 0.97	1.94 ± 0.67	100

B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts

B:W – Biomass : Water in 1:8.5 parts

* Others minor components not included

** Mass of dry biomass after pretreatment

Table 2
Composition Analysis of non-soaked energy cane mixtures after pretreatment

Pretreatment Conditions				Biomass component* (% dry weight)				Recovered solids**
Mixtures	Time (min)	Power (Watt)	Frequency (KHz)	Lignin	Glucan	Xylan	Arabinan	
B:A:W	30	200	20	23.75 ± 0.72	43.77 ± 1.69	25.04 ± 1.79	2.17 ± 0.34	88.22 ± 0.32
	45			26.48 ± 2.76	45.44 ± 1.27	24.32 ± 1.71	2.13 ± 0.54	87.20 ± 0.29
	60			26.86 ± 0.79	50.93 ± 2.47	25.75 ± 2.05	2.15 ± 0.50	86.40 ± 0.13
B:A:W	30	200	0 (OFF)	22.86 ± 0.26	30.65 ± 0.04	17.76 ± 0.35	1.2 ± 0.11	82.96 ± 0.06
	45			22.5 ± 0.64	30.46 ± 0.66	17.81 ± 0.56	1.23 ± 0.04	82.10 ± 0.29
	60			23.05 ± 0.28	32.01 ± 0.25	17.71 ± 0.25	1.21 ± 0.14	83.95 ± 0.08
B:W	30	200	20	25.02 ± 1.09	32.66 ± 0.88	18.55 ± 1.70	1.9 ± 0.78	88.71 ± 0.30
	45			26.97 ± 2.05	32.77 ± 0.33	17.75 ± 0.18	1.05 ± 0.16	88.27 ± 0.38
	60			29.45 ± 1.26	34.14 ± 0.89	18.15 ± 0.42	0.98 ± 0.08	88.99 ± 1.41
B:W	30	200	0 (OFF)	23.85 ± 0.40	23.03 ± 0.07	12.68 ± 0.45	0.88 ± 0.02	85.09 ± 0.16
	45			26.02 ± 0.17	23.94 ± 0.09	12.16 ± 1.13	0.88 ± 0.01	86.04 ± 0.78
	60			28.95 ± 0.56	25.74 ± 0.86	13 ± 0.14	0.98 ± 0.08	86.91 ± 0.51
Untreated energy cane				25.71 ± 0.59	40.48 ± 0.69	21.51 ± 1.93	1.92 ± 0.23	100

B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts

B:W – Biomass : Water in 1:8.5 parts

* Others not included

** Mass of dry biomass after pretreatment

Table 3

Glucan and xylan loss, lignin removal and glucose yields for non-soaked samples(using simultaneous enzymatic hydrolysis)

Mixtures	Reaction time (Minutes) / Frequency (kHz)	% Glucan loss	% Xylan loss	% Lignin removed	% Glucan	% Glucose yield after 72 hours	Ethanol yield g / 100g dry biomass
B:A:W	30 / 20	7.99 ± 0.09	7.63 ± 0.57	9.88 ± 0.32	43.77 ± 1.69	34 ± 0.02	16.94 ± 0.96
	45 / 20	1.48 ± 0.14	12.04 ± 0.07	13.89 ± 0.94	45.44 ± 1.27	42 ± 0.02	22.34 ± 0.57
	60 / 20	0.32 ± 0.01	11.37 ± 0.63	23.83 ± 0.93	50.93 ± 2.47	44 ± 0.00	24.29 ± 0.09
	30 / 0	38.03 ± 0.15	36.06 ± 0.49	26.06 ± 1.01	30.65 ± 0.04	30 ± 0.02	11.82 ± 0.64
	45 / 0	37.48 ± 0.51	36.92 ± 0.54	26.26 ± 0.38	30.46 ± 0.66	34 ± 0.00	13.44 ± 0.15
	60 / 0	33.76 ± 0.31	35.11 ± 0.70	23.94 ± 0.13	32.01 ± 0.25	35 ± 0.01	13.78 ± 0.39
B:W	30 / 20	49.56 ± 0.81	51.03 ± 1.61	16.26 ± 0.91	32.66 ± 0.88	17 ± 0.00	8.25 ± 0.05
	45 / 20	29.06 ± 0.86	30.77 ± 0.73	11.62 ± 0.70	32.77 ± 0.33	20 ± 0.01	8.89 ± 0.41
	60 / 20	26.83 ± 0.24	32.36 ± 0.73	11.76 ± 0.19	34.14 ± 0.89	22 ± 0.01	10.07 ± 0.38
	30 / 0	51.22 ± 1.09	52.97 ± 1.38	19.50 ± 0.70	23.03 ± 0.07	17 ± 0.00	6.47 ± 0.01
	45 / 0	48.70 ± 1.15	49.78 ± 1.63	13.78 ± 0.37	23.94 ± 0.09	20 ± 0.00	7.32 ± 0.18
	60 / 0	47.20 ± 1.09	49.17 ± 1.20	2.98 ± 0.10	25.74 ± 0.86	20 ± 0.01	7.69 ± 0.24

All values are in dry basis

B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts

B:W – Biomass : Water in 1:8.5 parts

Table 4

Composition Analysis of soaked energy cane mixtures after pretreatment

Pretreatment Conditions				Biomass component* (% dry weight)				Recovered solids**
Mixtures	Time (min)	Power (Watt)	Frequency (KHz)	Lignin	Glucan	Xylan	Arabinan	
B:A:W 3Hrs soaked	30	200	20	23.10 ± 0.46	42.65 ± 0.53	24.13 ± 0.63	3.07 ± 0.11	87.20 ± 0.29
	45			20.92 ± 0.07	45.61 ± 0.39	24.77 ± 0.23	3.39 ± 0.18	85.20 ± 0.30
	60			20.05 ± 0.16	47.65 ± 0.27	25.04 ± 0.12	3.70 ± 0.19	85.12 ± 0.54
B:A:W 3Hrs soaked	30	200	0 (OFF)	21.96 ± 0.51	42.9 ± 0.31	22.37 ± 0.48	3.46 ± 0.05	81.86 ± 0.21
	45			22.72 ± 0.28	42.40 ± 0.29	20.26 ± 0.74	2.88 ± 0.33	80.52 ± 0.69
	60			22.30 ± 0.36	40.56 ± 0.44	19.28 ± 0.16	2.52 ± 0.20	81.00 ± 0.30
B:W 3Hrs soaked	30	200	20	26.73 ± 0.55	29.98 ± 0.06	22.09 ± 0.38	2.29 ± 0.00	90.59 ± 0.12
	45			25.83 ± 0.28	35.73 ± 0.28	19.81 ± 0.71	1.50 ± 0.02	90.79 ± 0.39
	60			22.66 ± 0.30	38.12 ± 0.20	17.00 ± 0.10	1.49 ± 0.01	91.82 ± 0.54
B:W 3Hrs soaked	30	200	0 (OFF)	22.44 ± 0.45	30.81 ± 0.19	16.66 ± 0.34	1.83 ± 0.07	86.56 ± 0.49
	45			22.78 ± 0.73	33.07 ± 0.43	17.49 ± 0.51	1.85 ± 0.05	87.3 ± 0.42
	60			23.29 ± 0.62	33.22 ± 0.63	19.15 ± 0.16	2.05 ± 0.07	89.84 ± 0.37
Untreated energy cane				25.71 ± 0.59	40.48 ± 0.69	21.51 ± 1.93	1.92 ± 0.23	100

B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts

B:W – Biomass : Water in 1:8.5 parts

* Others not included

** Mass of dry biomass after pretreatment

Table 5

Glucan and Xylan loss, lignin removal and glucose yields for soaked samples (using simultaneous enzymatic hydrolysis)

Mixtures	Reaction time (Minutes) / Frequency (kHz)	% Glucan loss	% Xylan loss	% Lignin removed	% Glucan	% Glucose yield after 72 hours	Ethanol yield g / 100g dry biomass
B:A:W SOAK	30 / 20	7.43 ± 0.60	5.01 ± 0.51	20.10 ± 0.28	42.65 ± 0.53	21 ± 0.01	11.89 ± 0.30
	45 / 20	5.69 ± 0.65	8.49 ± 0.39	30.53 ± 0.77	45.61 ± 0.39	38 ± 0.01	20.10 ± 0.47
	60 / 20	0.81 ± 0.08	6.11 ± 0.94	33.81 ± 0.84	47.65 ± 0.27	44 ± 0.01	23.99 ± 0.52
	30 / 0	14.81 ± 0.32	18.47 ± 0.21	28.29 ± 0.42	42.90 ± 0.31	37 ± 0.01	17.96 ± 0.64
	45 / 0	17.33 ± 0.53	30.81 ± 1.25	29.66 ± 0.64	42.40 ± 0.29	37 ± 0.01	17.80 ± 0.03
	60 / 0	20.15 ± 0.43	31.66 ± 0.63	28.04 ± 0.55	40.56 ± 0.44	38 ± 0.0	18.24 ± 0.05
B:W SOAK	30 / 20	32.74 ± 1.25	10.53 ± 0.77	3.21 ± 1.07	29.98 ± 0.06	20 ± 0.00	8.71 ± 0.16
	45 / 20	20.75 ± 0.61	22.96 ± 1.39	10.26 ± 0.37	35.73 ± 0.28	21 ± 0.00	9.92 ± 0.01
	60 / 20	14.20 ± 0.36	31.93 ± 0.42	18.00 ± 0.49	38.12 ± 0.20	22 ± 0.02	10.91 ± 0.54
	30 / 0	34.62 ± 0.55	37.24 ± 0.99	22.13 ± 0.70	30.81 ± 0.19	22 ± 0.02	9.34 ± 0.71
	45 / 0	30.00 ± 0.24	34.29 ± 0.90	19.22 ± 0.95	33.07 ± 0.43	27 ± 0.01	11.41 ± 0.43
	60 / 0	25.65 ± 0.42	24.04 ± 0.46	17.07 ± 0.54	33.22 ± 0.63	28 ± 0.01	11.97 ± 0.64

All values are in dry basis

B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts

B:W – Biomass : Water in 1:8.5 parts

Table 6

Theoretical ethanol yield and energy produced for non-soaked and soaked pretreated sample mixtures

			NON-SOAKED SAMPLES			SOAKED SAMPLES	
Mixtures	Reaction time (Min) / Frequency (kHz)	Process energy input (kJ)	Ethanol from 100g dry biomass	Energy produced per 100 g dry biomass (kJ)		Ethanol from 100g dry biomass	Energy produced per 100 g dry biomass (kJ)
B:A:W	30 / 20	756.00	16.94 ± 0.96	503.11		11.89 ± 0.30	523.31
	45 / 20	1044.00	22.34 ± 0.57	663.49		20.10 ± 0.47	768.92
	60 / 20	1368.00	24.29 ± 0.09	721.41		23.99 ± 0.52	886.34
	30 / 0	396.00	11.82 ± 0.64	351.05		17.96 ± 0.64	697.37
	45 / 0	504.00	13.44 ± 0.15	399.16		17.80 ± 0.03	691.76
	60 / 0	648.00	13.78 ± 0.39	409.26		18.24 ± 0.05	707.02
B:W	30 / 20	756.00	8.25 ± 0.05	245.02		8.71 ± 0.16	434.83
	45 / 20	1044.00	8.89 ± 0.41	264.03		9.92 ± 0.01	479.12
	60 / 20	1368.00	10.07 ± 0.38	299.07		10.91 ± 0.54	513.83
	30 / 0	396.00	6.47 ± 0.01	192.15		9.34 ± 0.71	448.3
	45 / 0	504.00	7.32 ± 0.18	217.4		11.41 ± 0.43	522.22
	60 / 0	648.00	7.69 ± 0.24	228.39		11.97 ± 0.64	545.37