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Use of ultrasound in the production of bioethanol from lignocellulosic biomass

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Abstract

Production of bioethanol from lignocellulosic biomass inevitably requires the biomass to be pretreated to remove lignin and produce fermentable sugars. This review is focused on the potential of ultrasound for improving the production of lignocellulosic bioethanol. Sonication can improve biomass pretreatment processes, the conversion of cellulose to fermentable sugars and also the fermentation step. Sonication can supplement existing biomass pretreatment methods to improve their efficacy and economics, or it can be used on its own. Cellulose hydrolysis to sugars can be improved with sonication. While overzealous sonication can be damaging to enzymes and cells, it can be effectively used to enhance enzymatic treatments and fermentation of sugars to bioethanol. Factors affecting sonicated processing of the lignocellulosic biomass to bioethanol are discussed.

Keywords: Ultrasound; Sonication; Lignocellulose; Bioethanol; Cellulose; Biofuels

1. Introduction

Bioethanol is perhaps one of the most successful biofuels [1]. It has at least partly substituted petroleum-based transport fuels. Bioethanol is produced by fermentation of sugars derived mainly from sugarcane and various starchy crops. Corn, potatoes and cassava are examples of some such crops. The use of these food and feed crops for producing biofuels is contentious. Potentially, bioethanol can be produced by fermentation of sugars derived from hydrolysis of the lignocellulosic plant biomass [2-3]. Production of fermentable sugars from lignocellulosic biomass is challenging and much effort is being invested in making it economically feasible. Use of lignocellulosic biomass as a feedstock can greatly increase the volume of bioethanol production, and it may eliminate its dependence on food crops. Lignocellulosic biomass derived from plants is abundantly available and its supply is renewable. Annually about 1×10^{10} metric tons of lignocellulosic biomass is produced worldwide [3-4].

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Lignocellulosic biomass consists mainly of cellulose, hemicellulose and lignin [5]. The specific composition depends on the source of the biomass. The arrangement of these components inside the biomass makes it extremely complex structure [3]. Only cellulose and hemicellulose can be converted to fermentable sugars. Recovery of these components from the biomass requires some kind of pretreatment [6-8]. A pretreatment process breaks down the lignin surrounding the cellulose molecules (Fig. 1). The hemicellulose polymer that binds the cellulose molecules together into fibers is generally broken down by pretreatment operations. Pretreatment may also break some of the cellulose molecules particularly in the amorphous regions. Removal of the lignin and hemicellulose allows hydrolytic reagents an improved access to the cellulose molecules in the subsequent hydrolysis steps [2-3].

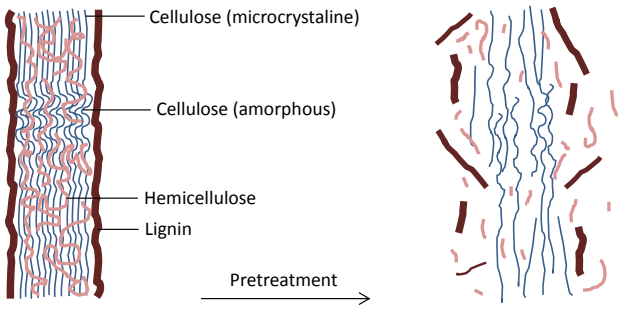

Fig. 1. Effect of a pretreatment operation on lignocellulosic fibers. Adapted from Kumar et al. [11] and Hsu et al. [137].

An extremely wide variety of pretreatment processes have been used. These include physical pretreatments (e.g. size reduction, steaming, hot water, steam explosion), chemical methods (e.g. acid, alkali, ionic liquids), biological treatments and their combinations [3, 9-12]. No single pretreatment has become established as a preferred method [3], partly because the lignocellulosic biomass from different sources is different. A pretreatment method is intended to make cellulose and hemicellulose more amenable to processes that are used to convert these polymers to fermentable sugars [2, 5, 7].

The use of ultrasound for pretreating lignocellulosic biomass is the focus of this review. Ultrasound is already used to improve a variety of bioprocesses [13-17] and has the potential to be used in producing ethanol from lignocellulosic materials. Ultrasonication is a potential alternative to conventional pretreatments of lignocellulosic biomass [18], or it can supplement an otherwise conventional pretreatment to greatly enhance its performance.

2. Ultrasound and the sonication process

Sonication of a biomass slurry is a physicochemical treatment process that provides energy in the form of sound waves. Ultrasound is sound of frequency greater than 16-20 kHz [19]. High-energy, or ‘power ultrasound’ has the frequency range of 16–100 kHz. Ultrasound ranging in frequency from 100 kHz to 1 MHz is regarded as ‘high frequency ultrasound’. Low energy ‘diagnostic ultrasound’ has the frequency range of 1–10 MHz [20]. Only power ultrasound is sufficiently energetic to be used in biomass pretreatment processes, but low-intensity sonication has the potential to improve the conversion of sugars to ethanol in the fermentation step. Ultrasound is transmitted to a fluid typically via a sonic horn, or sonotrode, or some other form of a sound transducer. A sonic horn generally consists of a solid rod that oscillates along its vertical axis at the frequency of ultrasound. The tip of the horn is

immersed in the medium being sonicated (Fig. 2) to carry out direct sonication. Direct sonication transfers sonic energy directly to the fluid being processed and is best for processing slurries of lignocellulosic biomass [13]. In fermentation processes involving live cells, indirect sonication may be effective. In this form of sonication, the sonic horn or transducer imparts energy to a bath of fluid surrounding the vessel containing the material being processed [21].

Fig. 2. A sonic horn, or sonotrode, immersed in a liquid for sonication. Courtesy of Qsonica LLC, Newtown, CT, USA.

Sonication produces a rapid cycling of pressure. During rarefaction phase of sonication, the low pressure results in the formation and growth of cavitation microbubbles in the liquid being sonicated [22]. During the subsequent compression phase, the bubbles implode releasing energy in the form of shock waves that propagate through the fluid. Collapse of cavitational bubbles causes a rapid rise in local temperature and pressure [13-14, 23]. Local temperature and pressure may rise to 10,000 K and 5,000 bar, respectively [14, 22]. Shock waves produce acoustic streaming, or movement of the fluid in the direction of propagation of the sound wave. Acoustic streaming is caused by the interaction of the sound wave with the molecules of the fluid. Streaming causes intense microscale turbulence [24], dissipating the acoustic energy. Pressure and heat effects may lead to the generation of free radicals [25-26] to promote certain reactions. Turbulence enhances mixing and mass transfer at the solid–liquid interface. Properties of the fluid influence the bubble collapse and cavitational effects [27]. Dissipation of acoustic energy may induce motion at the level of molecules, particularly macromolecules, particles and suspended cells. Erosion of solid material is promoted [28]. Thus, ultrasonication produces both physical and chemical effects in the slurry being sonicated [14, 29-32]. All these factors contribute to the pretreatment of lignocellulosic biomass.

Numerous studies have already confirmed the efficacy of ultrasound as a pretreatment of lignocellulosic biomass [18, 33-42]. Sonication has also been shown to facilitate hydrolysis of cellulose and hemicellulose to sugars. Fermentation of sugars to bioethanol can also be improved by using ultrasound [21].

3. Effects of sonication on biomass particles

The physiochemical effects of sonication alter the morphology of lignocellulosic biomass particles suspended in a fluid [28, 43]. Cavitation causes significant disruption of the biomass

in a suspension. Asymmetric bubble collapses close to the solid–liquid interface generates liquid jets that fragment particles and increase their surface area [32, 44]. Sonication has been shown to modify the surface of soy flake biomass by creating microfractures [45]. In one study, the size of sorghum biomass in slurry could be reduced by 50% using sonication [44]. Ultrasonication of cellulose fibers results in partial fibrillation, or peeling of fibrils from the surface of the fibers, causing the fibers to separate. Weaker interfaces are disintegrated [46] and this increases the surface area for action of cellulose hydrolytic enzymes to facilitate production of fermentable sugars [36, 47-48]. Sonication pretreatment of water hyacinth biomass disrupted cell walls and made the biomass more amenable to downstream processes for producing sugars. This process increased the sugar yield from a given amount of biomass [41, 48].

Sonication causes homolysis of lignin–carbohydrate bonds to release lignin and hemicellulose [26, 49-50]. The aromatic rings in the lignin are opened up at the α-position by cleavage of the C–C bonds [51]. The C–H bonds are also susceptible to disruption. The disruption of bonds leads to the formation of macroradicals. These macroradicals, together with the ·H and ·OH radicals produced by cavitation, stimulate the depolymerization of the lignocellulosic material. Depolymerization of starchy material by sonication has also been reported extensively in the literature [51-55]. The cavitation phenomenon associated with sonication has been shown to disrupt bonds within polymers. In one study, sonication reduced the molecular weight of starch to 4% of the initial weight [56]. Unlike some of the other pretreatment methods [49], sonication does not alter the chemical composition of the lignocellulosic biomass [57]. The ultrasound-induced structural changes in biomass depend on the sonication power and duration.

4. Factors affecting sonication

Efficacy of a sonication pretreatment depends on a number of factors. The cavitation intensity and the active cavitational volume within a treatment vessel are two important factors. These in turn are affected by the duration of sonication, processing temperature, frequency of ultrasound, sonication power, amplitude of sonication, efficiency of the ultrasound transducer, properties of the suspension, and properties of the biomass. The configuration of the sonication reactor also influences efficacy of sonication process [14, 58-60]. Some of the major factors affecting the sonication process are discussed.

4. 1. Sonication duration

For otherwise fixed conditions, the duration of the sonication has the greatest impact on the pretreatment achieved. Increased duration of sonication increases delignification of the biomass and release of sugars from it, but prolonging sonication beyond a certain duration produces no added benefit in terms of delignification and sugar release [34, 55, 57, 61-63]. A less recalcitrant starchy biomass requires a short sonication treatment, e.g. of the order of minutes, compared to a more recalcitrant lignocellulosic biomass. A 40 s sonication of a corn starch slurry was sufficient to increase the sugar yield by 5- to 6-fold compared to control [24]. In a different study, the glucose concentration after a 5 min treatment of corn starch was increased by only about 3% compared to control [62]. Clearly, the sonication regimen and the other operational factors affect the efficacy of sonication. Starch particles consist of both ordered crystalline regions and amorphous regions. The amorphous portion of the starch is more readily solubilized by ultrasonic action compared to the crystalline portion [55].

A sonicated alkaline pretreatment (15-35 min) increased delignification of wheat straw by 7.6-8.4% compared to the non-sonicated alkaline pretreatment. This improvement was

attributed to the hydrodynamic shear forces associated with sonication. A shorter sonication treatment of 5-10 min in combination with the alkali treatment did not achieve any significant delignification compared with non-sonicated alkali pretreatment [34]. Similar results have been reported for ultrasonic pretreatment of wheat straw in an organic solvent. The solubilization of lignin and hemicellulose increased with increasing the duration of sonication for up to 25 min, but further prolonging the sonication treatment up to 35 min produced no additional benefit. The effect of sonication was attributed to an improved contact between the solvent and the suspended solids, and improved mass transfer [61].

Ultrasound-assisted extraction of xylan from corn cobs has been described in the literature [64, 65]. Increasing the duration of sonication from 10 to 60 min improved the release of xylan from corncob biomass from around 10% to 30% [66]. Wang and Zhang [65] reported on the effect of sonication on xylan release from corncob biomass suspended in an alkaline medium. The xylan release rate increased with increasing duration of sonication for up to 30 min, but no improvement was observed thereafter [65]. Sugar release from water hyacinth biomass also increased as a function of sonication time [41]. Yunus et al. [39] reported a xylose recovery of 52% from oil palm empty fruit bunch fiber biomass following a 45 min sonication treatment. A relatively short span sonication has also been preferred for converting grain starch to glucose [12, 43-44, 55, 67-68].

4. 2. Sonication power and amplitude

A sonication process is strongly influenced by the ultrasound power level, as the power intensity directly affects the cavitation phenomena. However, at any power level, prolonging the treatment duration beyond a certain time does not generally improve sugar release from the biomass [24, 63]. Therefore, for a particular biomass and slurry characteristics, the sonication regimen (i.e. power level and treatment duration) needs to be optimized beforehand to meet the specified pretreatment objectives. Excessive sonication may turn a process into being uneconomic, and may lead to the formation of byproducts. The energy demand per gram of sugar released has been found to increase with increasing time of sonication [24]. Sonication power influences the number of cavitation bubbles produced, their lifetime and the implosion pressure [60]. In a given sonication treatment, the sonication power and the processing time required are generally inversely correlated [36]; thus, increasing the sonication power would generally shorten the irradiation period required for a given treatment objective. In cellulase mediated hydrolysis of cellulose, the time t needed for sonication has been correlated with the irradiation power P, as follows: $t \propto P^{-1.55}$ [36].

Sonication process can adversely affect the outcomes at higher sonication power level. Number of bubbles may form near the tip of the ultrasound transducer at a high power level, which impede the transfer of energy from the transducer to the liquid medium [60]. Aimin et al. [18] reported on increased oxidation of cellulose as the sonication power was increased to 400 W in 200 mL of the slurry. A suspension of poplar wood cellulose powder was transformed to a viscous suspension when sonication power was raised to 1200 W [46] in 120 mL of the slurry, but the cellulose walls of the plant cells were not completely disrupted. Within limits, increasing the sonication power has increased xylan release from corncob biomass [64, 66].

Amplitude of a sound wave is the distance between the adjacent peaks of the wave. An increased amplitude of sonication has been associated with an improved sugar yield in processing of oil palm empty fruit bunch fibers [39], but contradictory findings have also been reported. A lower amplitude ultrasound has been claimed to improve the yield of protein and sugar on a per unit energy basis compared to sonication at a higher amplitude [45]. In

liquefaction of spruce wood meal, the time required depended on the amplitude of the sound. Liquefaction time was shortened from 120 min to 80 min by increasing the sonication amplitude from 20% to 80%. Furthermore, liquefaction was accomplished within 10 min at a sonication amplitude of 100% at an energy intensity of 85 W cm^{-2} [25].

The duration of sonication was suggested to be more important than the amplitude of sonication in processing of slurries of oil palm empty fruit bunch fibers [39]. In ultrasonic processing of corn biomass, medium power settings have been reported a better option compared to high power settings [24].

4. 3. Frequency of ultrasound

The frequency of ultrasound influences its power level. High-energy, or 'power ultrasound' has a frequency range of 16–100 kHz. This frequency range is commonly used in biomass processing scenarios. Low energy 'diagnostic ultrasound' has the frequency range of 1–10 MHz [20] and is generally not considered useful in most of the bioprocessing situations.

The selection of ultrasound frequency for a given application depends on the nature of process. An ultrasound frequency range of 10–100 kHz has been recommended for processes that require intense physical effects such as cell breakage and polymer degradation [60]. This frequency range would be applicable in nonenzymatic and nonmicrobial ultrasonic processing of lignocellulosic particles. Within the frequency range associated with power ultrasound, increasing the frequency of ultrasound waves is not automatically beneficial. Treatment of recalcitrant biomass requires more energetic ultrasound compared to treatment of a less recalcitrant starchy biomass. Processes involving enzymes and live cells require a milder sonication regimen [59, 60].

High-frequency sonication of lignocellulosic fiber suspensions at 610 kHz (100 or 200 W in ~450 mL, 3 h) was shown to modify only the surface structure of the fibers [69]. Multi-frequency operation can be used to enhance cavitational activity, and to improve energy efficiency in ultrasonic processing [60]. Dual frequency operations have been discussed in detail elsewhere [59, 60]. The synergetic effect of dual frequencies increases the number of cavitational events and the interactions among the bubbles formed to produce secondary turbulence effects [70]. Design considerations for multi-frequency sonochemical reactors have been discussed in the literature [71-73].

4. 4. Temperature

The energy of ultrasound is ultimately dissipated as heat within the suspension. Therefore, the temperature of the slurry usually rises in uncontrolled settings. An excessive rise in temperature can adversely affect certain biological processes, but in other cases control of temperature during sonication may not be necessary. Elevated temperature facilitates the enhanced reaction rate in case of acid and alkali pretreatment of the biomass. This effect can be used to integrate sonication with chemical pretreatment (acid and alkali) in order to make them workable at low temperature level. However, if a process involves enzymatic catalysis or microbial action, a control of temperature would be generally necessary to prevent deactivation of the biocatalyst. How much the temperature rises in a given processing situation of course depends on the rate of input of sonication energy per unit mass of the fluid [24, 63].

In the absence of control, the temperature rose to ~60 °C within 30 s during power sonication (8 W/mL) of cassava biomass chips [74]. Similarly, sonication of defatted soy

flakes slurry raised the temperature of the slurry (500 mL) to around 38 °C within 120 s at a sonication power density of 2.65 W/mL. These processes did not involve any enzymes, thus, the above mentioned levels of temperature rise did not reduce the yield of fermentable sugars from the biomass. Use of sonication substantially improved the yield of sugars and proteins from soy flakes relative to nonsonicated control treatment [45].

The dynamics of the sonication-induced cavitation are apparently not affected by changes in temperature over the range of 20-60 °C [73]. An elevated temperature simply increases the rates of the chemical reactions contributing to biomass pretreatment. Too high a temperature rise may be counterproductive. The collapse of the cavitational bubbles during the compressive phase of sonication may be slowed by an increased operating temperature. Bubble collapse, or implosion, is associated with the generation of the shock waves that are ultimately responsible for the myriad effects of ultrasound. An increased temperature reduces the solubility of gases in a liquid and this too adversely affects cavitation. Therefore, too large an increase in temperature has the potential to adversely impact the sonication process [24, 59].

In comparison with operation at room temperature, glucose release from corn meal could be increased by increasing the sonication process temperature to 60 °C. At 60 °C, 105 g/L of glucose was released from corn meal after 5 min of sonication [62]. Similar observations have been reported for wheat straw [61]. During sonicated saccharification of triticale meal suspensions, increasing temperature from 40 °C to 60 °C increased the release of glucose and maltose [68], but this may have been an effect of temperature on the rate of the enzymatic reaction and not a possible effect of temperature on the efficacy of sonication. Good release of sugars through high power sonication has been reported from corn also at relatively low processing temperatures of 30–40 °C [43]. Thus, the effect of temperature on sonication appears to depend on the type of biomass and the other process parameters. The yield of glucose is generally increased by sonication compared to controls, but the extent of increase can significantly depend on the biomass being processed and the other operational factors of the sonication process [62].

4. 5. The liquid medium

The characteristics of the liquid, used to suspend the lignocellulosic particles, affect the sonication process. Mostly, the liquid is an aqueous solution. However, organic solvents and ionic liquid are also used in biomass pretreatment. The physicochemical properties of the medium (density, vapor pressure, viscosity and surface tension) influence the propagation of sound waves, cavitational process, transport phenomena and the generation of radicals [60, 75]. Liquids with a high vapor pressure and a high viscosity are less amenable to cavitation. Liquids having a high surface tension tend to produce a more intense shock wave as the cavitation bubbles implode [60]. A high viscosity attenuates sound waves to reduce the zone of active cavitation. An increased density of the fluid reduces attenuation of the sound wave. In addition to being satisfactory for sonication, a medium for processing lignocellulosic biomass must be inexpensive and nontoxic. Water and dilute aqueous solutions of inorganic alkalis and acids are the usual media.

Distilled water has been used in sonication processing of sunflower seed husk [49], but there is little rationale for preferring it over tap water. Water has a relatively low energy threshold of cavitation. Sonication of water generates hydronium ion (H_3O^+) and hydroxyl radicals (·OH) that act as oxidizing agents to enhance the rate of depolymerization of suspended biomass [76]. These reactive species in combination with the local high pressure and temperature generated by sonication facilitate the destruction of the lignin–carbohydrate

bonds [26, 49, 76].

For pretreatment of lignocellulosic biomass, García et al. [28] compared three different liquid media, water, aqueous acetic acid (60% v/v) and aqueous NaOH (7.5% w/w). The efficacy of pretreatment in water was comparable to the efficacy in aqueous acetic acid; however, the pretreatment in the aqueous alkali medium took twice as long as the treatment in water for attaining the same yield of the hydrolysate [75].

4. 6. Suspended solids

Dynamics of sonication are affected by the presence of suspended solids compared to the case of only a liquid being processed. Pretreatment of lignocellulosic biomass necessarily involves slurry at least in the early stages of the pretreatment process. For reasons of cost, the use of concentrated slurry is often preferred over dilute suspension. In slurry, the cavitational activity is enhanced by the presence of the solids, but a high concentration of solids inevitably increases viscosity to reduce mixing, mass transfer and heat transfer. Therefore, the concentration of the suspended solids needs to be selected as a compromise between the effects of an enhanced cavitation activity and reduced transport phenomena [59, 60]. The size of the lignocellulosic particles also influences the extent and the severity of sonication treatment needed to achieve a given objective. Size reduction is itself an energy intensive operation, and therefore, there is a scope for optimizing the particle size in relation to subsequent ultrasonic processing.

5. Ultrasound mediated production of lignocellulosic bioethanol

Sonication may be used in all the main stages of production of bioethanol from lignocellulosic biomass. These production stages include biomass pretreatment for delignification and improved hydrolysis; the hydrolysis of cellulose and hemicellulose to fermentable sugars; and the fermentation of sugars to bioethanol. Table 1 summarizes the literature pertaining to the use of ultrasound in various facets of production of bioethanol. Sonicated processing in the above mentioned production stages is discussed in the following sections.

Table 1. Use of ultrasonication for biomass pretreatment, hydrolysis, and fermentation

Application	Biomass	Liquid medium	Sonication settings	Results	Reference
Pretreatment	Eucalyptus cellulose fiber	Sodium periodate	23-25 kHz, 30 s	Cellulose accessibility increased without much change in its structure	[18]
Pretreatment	Poplar wood	Distilled water	20-25 kHz, 400-1200 W	Hemicellulose and lignin were removed	[46]
Pretreatment	Olive tree pruning residues	Acetic acid, sodium hydroxide, water	420 W, 50-60 kHz, 30-120 min, 50 °C	Hemicellulose and lignin were removed. Sonication in water and acetic acid produced similar results. A longer treatment was required with alkali	[28]
Pretreatment	Water hyacinth	Distilled water	20 kHz, 20-100% power, 10-30 min, 30 °C	Sonication at 20 min and 100% irradiation power yielded 133 mg sugar/g dry matter	[41]
Pretreatment	Carboxymethyl cellulose	Acetate buffer	135 W, pH 4.8, 323 K	Sonication improved subsequent enzymatic hydrolysis rate	[36]
Pretreatment	Hexane-defatted soybean flakes	Tap water	2.2 kW, 20 kHz, amplitude level 144 μm, power density 0.3-2.56 W/mL, 15-120 s	Sonication at high amplitude for 120 s reduced particle size by 10-fold and increased total sugar released by 50%	[45]
Pretreatment	Extruded full-fat soybean flakes	Sodium acetate buffer and water	2.2 kW, 20 kHz, 21-84 μm, 30-60 s	Non-significant improvement in saccharification	[8]
Pretreatment	Corn slurry	Acetate buffer and water	2.2 kW, 20 kHz, 24-299 μm, 20-40 s	Sonication at high amplitude for 120 s reduced particle size by 20-fold and increased total sugar	[43]

				release by 3-fold	
Pretreatment	Medium density fiberboard, veneered particleboard, oriented strand board, plywood, wheat straw	Water	400 W, 24 kHz, amplitude 20-100%	Sonication reduced the reaction time by 9-fold	[25]
Pretreatment	Sugarcane bagasse	N-methylmorpholine-N-oxide	45 kHz, 100 W, 90 °C	Sonication resulted in 96.5% hydrolysis of biomass	[50]
Pretreatment	Corn	Acetate buffer, distilled water	20 kHz, 192-320 µm, 5-40 s	Sonication increased sugar release by 300%	[24]
Pretreatment	Corn		21 kHz, 192-320 µm, 20-40 s	Sonication increased sugar release by 2-3 fold relative to control	[63]
Pretreatment	Corn	Water	40 kHz, 600 W, 60-80 °C, 1-30 min	Sugar yield was increased by up to 7%	[62]
Pretreatment	Corn		41 kHz, 600 W, 60 °C, 1-10 min	Bioethanol yield was increased by up to 11 %	[12]
Pretreatment	Kenaf core fiber	Various ionic liquids	24 kHz, 35 W, 25 °C, 0-120 min	Sonication improved glucose yield up to 60-95% in all ionic liquids compared to heat pretreatment	[86]
Pretreatment	Cassava chips	Acetate buffer	20 kHz, 2.2 kW, 80-320 µm, 2-8.5 W/mL, 10-30 s	Sonication increased bioethanol yield 2.7-fold relative to control and reduced fermentation time by 24 h	[67]
Pretreatment	Triticale	Water	40 kHz, 125 W, 40-60 °C, 5 min	Sonication improved glucose yield by 15.7% and bioethanol yield by up to 11%	[68]
Pretreatment	Sugarcane bagasse	Sodium hydroxide	24 kHz, amplitude 100%, 50 °C, 20 min	Sonication removed about 75% of lignin. Recovery of hemicellulose and cellulose were up to 79% and 99%, respectively	[40]
Pretreatment	Sugarcane bagasse	Sodium hydroxide (2%)	25 kHz, 400 W, amplitude 100%, 50 °C, 20 min	Sonication removed nearly 81% of the lignin and up to 91% of the hemicellulose	[90]
Pretreatment	Sugarcane bagasse	Sodium hydroxide (2.9%)	25 kHz, amplitude 100%, 70 °C, 47 min	The predicted reducing sugar yield of the optimized sonicated process was of 97%	[91]
Pretreatment	Sorghum flour	Acetate buffer and $CaCl_2$	20 kHz, 750 W, 1 min	Sonication reduce particle size by 50% and increased saccharification by 8%	[44]
Pretreatment	Wheat straw	Potassium hydroxide	20 kHz, 100 W, 5-35 min, 35 °C	More than 50% of the lignin was removed	[34]
Pretreatment	Wheat straw	Sodium hydroxide, methanol and water	20 kHz, 100 W, 5-35 min, 60 °C	More than 9% of hemicellulose was extracted than non-sonicated biomass	[61]
Pretreatment	Sugarcane bagasse	Distilled water	22 kHz, 100 W, 5-35 min, 55 °C	Sonication allowed more than 90% extraction of hemicellulose and lignin	[92]
Pretreatment	Corncob	Sulfuric acid	22 kHz, 1 kW, 10-60 min, liquid to solid ratio of 10	Sonication could extract 39% of xylose with a 97% shortening of the processing time	[66]
Pretreatment	Microcrystalline cellulose	Alkylphosphate ionic liquids	45 kHz, 100 W, 60 °C, 30 min	Sonication resulted in 95.5% conversion of cellulose to glucose	[87]
Pretreatment	Rice hull	Not mentioned	40 kHz, 250 W, 10-60 min, 25 °C	Non-significant improvement in saccharification yield was found	[38]
Pretreatment	Poplar wood	Ethanol, methanol, dioxane, dimethyl sulfoxide, sodium hydroxide	20-24 kHz, 570 W, 30 min, 25 °C	Sonication followed by sodium hydroxide extraction could release 96% of lignin and 75.5% of hemicellulose	[78]
Pretreatment	Oil palm empty fruit bunch	Sulfuric acid	20 kHz, 2 kW, 15-60 min, amplitude 15-90%, 25 °C	A 3-fold increase in xylose yield was obtained with sonication	[39]
Pretreatment	Corn stover	Sodium hydroxide	4 kHz, 80 W, 25 °C	Sonication pretreatment reduced alkali requirement from 8% to 5% and the processing time was shortened by 2-fold relative to control. About 46% of lignin was removed	[37]
Hydrolysis	Paper pulp	Acetate buffer	20 kHZ, 250 W, 45 °C	Pulp was completely converted to sugar	[108]
Hydrolysis	Waste paper	Acetate buffer	30-60 W, 45 °C	Sonication effectively hydrolyzed waste paper except newspaper	[111]
Hydrolysis	Sugarcane bagasse	Sulfuric acid	24 kHz, amplitude 100%, 50 °C, liquid to solid ratio of 10-25/1, 15-75 min, acid	Bioethanol yield was 0.17 g/g and a lower concentration of inhibitors was produced	[40]

			concentration 1-3%		
Hydrolysis	Sugarcane bagasse	Distilled water	25 kHz, 400 W, 40 °C, 5 min with 5 min gap up to 3 h, *C. flavigena* 5-25 g/L, liquid-to-solid ratio of 10-25/1, pH 4-8, biomass loading of 5-25 g/L	Nearly 38 g/L of glucose was produced	[90]
Enzymatic hydrolysis	Sugarcane bagasse	N-methylmorpholine-N-oxide	45 kHz, 100 W, 50 °C,	Sonication resulted in 96.5% hydrolysis of biomass	[50]
Simultaneous saccharification and fermentation	Waste paper	Citrate buffer and water	36 kHz, 150 W, 7-60 min	Sonication increased bioethanol yield by 20 %. Continuous ultrasound exposure decreased ethanol production	[118]
Enzymatic hydrolysis	Corn stover	Water	20 kHz, 80 W, 55 °C	Cellulase activity was increased by 70%	[37]
Fermentation	Lactose	Dissolved nutrients in water	20 kHz, 15 W, 30 °C, duty cycles 10-40%, 1-2 s with 5 s rest period, *Kluyveromyces marxianus*	Sonication at 20% duty cycle resulted in a 3.5-fold increase in bioethanol productivity. Higher sonication power reduced cell growth	[21]

5. 1. Pretreatment of biomass

A variety of ultrasound assisted biomass pretreatment processes have been reported. All such processes have been developed by supplementing an otherwise conventional pretreatment with sonication, as discussed next.

5. 1. 1. Sonicated organic solvent treatment

Organic solvents have been used to delignify the lignocellulosic biomass by dissolving the lignin. Organic solvents target ether bonds such as α-aryl and arylglycerol-β-aryl ether bonds [61]. The conventional organic solvents based delignification, commonly referred to as the organosolv pretreatment, has been reviewed by Zhao et al. [77]. A successful coupling of sonication with organic solvents has been demonstrated for solubilizing lignin and hemicellulose from lignocellulosic biomass. Sonication (30 min) was used to treat dewaxed poplar wood in organic solvents (ethanol, methanol, dioxane) at neutral pH and 25 °C. This treatment delignified the biomass. The delignified biomass was further sonicated in the presence of dimethyl sulfoxide to extract hemicellulose under the above specified operational settings. More than 75% of the hemicellulose and >96% of the original lignin could be removed by this treatment [78].

Extraction of hemicellulose from wheat straw in a sonicated organic solvent system has also been reported [61]. Toluene–ethanol pretreated biomass suspended in NaOH–methanol–H_2O mixture was sonicated. Sonication, between 5 and 25 min, improved hemicellulose removal and delignification [61]. This effect was associated with an improved access of the solvent to the biomass under the sonicated conditions. Organosolv processes are generally considered too expensive for the production of bioethanol [77]. Therefore, a sonicated organosolv treatment is likely to be expensive compared to the other ultrasound supplemented treatments.

5. 1. 2. Sonicated ionic liquid treatment

Pretreatment of lignocellulosic biomass using ionic liquids is a potentially powerful emerging technology [79-80]. Ionic liquids consist entirely of ions, and are often able to

completely dissolve a large quantity of lignocellulosic biomass at ambient temperature [81, 82]. Ionic liquid can dissolve cellulose and reduce its crystallinity to improve its subsequent hydrolysis [83-84]. A great variety of lignocellulosic biomass is amenable to pretreatment with ionic liquids [79-80, 85]. Sonication in conjunction with ionic liquids offers other opportunities for biomass pretreatment.

Ultrasonic pretreatment of kenaf powder in different ionic liquids followed by a subsequent nonsonicated enzymatic hydrolysis, in the same ionic liquid as was used in the sonication treatment, resulted in up to 95% of cellulose being hydrolyzed in 120 min at 25 °C [86]. In contrast, thermal pretreatment (110 °C, 120 min) of the powder in ionic liquids released much less glucose [86]. Yang et al. [87] reported on the enzymatic saccharification of microcrystalline cellulose with ultrasonication in alkylphosphate types of ionic liquids. Depolymerization of cellulose was 3-fold greater in the sonicated ionic liquid treatment compared with control. In sonicated processing, a >90% conversion of cellulose to glucose was achieved within 8 h. In the absence of sonication, the conversion was <80% after 24 at 50 °C [87]. The processing time could be reduced applying sonication compared with non sonicated process. In view of these studies, sonication can be usefully combined with pretreatments involving ionic liquids.

5. 1. 3. Sonicated dilute acid treatment

Dilute acids pretreatment is highly effective process for lignocellulosic biomass [9, 11]. Although dilute inorganic acids have been widely used to pretreat lignocellulosic biomass, only a few studies of sonicated process with inorganic acids appear to exist. Yang et al. [66] used dilute sulfuric acid in sonicated pretreatment of corncob to extract xylan. Sonication improved xylan recovery rate and yield compared to the nonsonicated acid treatment. In sonicated processing, about 39% of xylan was extracted within 43 min. The conventional acid process took 24 h to extract 34% of xylan. In our lab studies, we found sonicated dilute acid hydrolysis of rice straw biomass to be highly effective. Sonication of olive tree pruning residues suspended in acetic acid (60% v/v) has been used to recover various components from this lignocellulosic material [28].

5. 1. 4. Sonicated alkaline treatment

Alkali pretreatment is another useful method that solubilizes lignin and improves digestibility of cellulose [88]. Integration of alkaline treatment with ultrasound has been shown to improve performance relative to nonsonicated treatment [89]. Sonicated treatments shorten processing time and reduce the requirement of alkali [37]. Ultrasound assisted alkali pretreatment of sugarcane bagasse has been described [40, 90, 91]. The rate of sugar release was positively affected by increasing the temperature, increasing the alkali concentration and reducing the particle size. Too high a concentration of solids in the slurry adversely affected the treatment as the slurry became difficult to mix [40, 90, 91]. For ultrasound-assisted alkali pretreatment of sugarcane bagasse, the optimal processing conditions have been reported to be: a particle size of about 0.3 mm, an initial solids concentration in the slurry of about 40 g/L, a sodium hydroxide concentration of 2.9%, and about 47 min of processing at 70 °C [91]. Between 75 and 90% of lignin was removed and the recovery of cellulose and hemicellulose was in the range of 79 to 99% [40, 90]. Other similar processes have been reported in literature [34, 65, 92]. Extraction of hemicellulose polysaccharides from buckwheat hulls in sonicated alkaline media has been reported [93].

Sonication treatment of wheat straw in an alkaline medium has been reported to disrupt the ether bonds between lignin and hemicellulose [34, 61], but without significantly affecting the composition and structure of lignin. Therefore, if pure lignin is wanted as a byproduct of a delignification process, sonication can be applied to some extent.

5. 2. Hydrolysis for production of sugars

After pretreatment, the biomass is generally subjected to either acid hydrolysis or enzymatic hydrolysis to release the fermentable sugars. Ultrasound can be usefully coupled to both the dilute acid hydrolysis and enzymatic saccharification to improve the rate of sugar release and the sugar yield. Processing time can be shortened through sonication.

5. 2. 1. Acid hydrolysis

Acid hydrolysis relies on an inorganic acid catalyst to hydrolyze the ether bonds [94]. Dilute sulfuric acid is generally used as it is less corrosive to process equipment compared to hydrochloric acid, for example. Dilute acid hydrolysis typically requires a temperature of >100 °C [95]. Ultrasound augmented sulfuric acid hydrolysis of sugarcane bagasse has been reported [40]. The process was sensitive to the biomass loading rate, the concentration of the acid and the duration of sonication. The optimal processing conditions were a liquid-to-solid mass ratio of 20:1, a 2% (g/100 mL) acid concentration and a sonication time of 45 min [40]. In our lab, ultrasound mediated acid hydrolysis has been used to simultaneously pretreat and hydrolyze the biomass. Sonicated processing at 80 °C using a 10% concentration of sulfuric acid yielded nearly 32 g of glucose per 100 g of rice straw within 50 min.

5. 2. 2. Enzymatic hydrolysis

Whereas power sonication is suitable for intensifying chemical pretreatments of lignocellulosic biomass, a less intense sonication regime is needed for enzymatic pretreatment and cellulose saccharification processes [13]. Cellulases are the enzymes most commonly used for the hydrolysis of pretreated biomass. The role of cellulases in deconstruction of the lignocellulosic biomass has been reviewed elsewhere [96]. The efficacy of cellulases is affected by many factors including the surface area of the biomass available for enzymatic action [97]. The biomass slurry needs to be mixed, as the enzyme must be continually adsorbed and desorbed at the surface of the biomass for rapid hydrolysis [98, 99].

Effects of ultrasound on enzymes have been thoroughly reviewed in the literature [13, 15]. Pressure fluctuations that constitute of an ultrasonic wave can dynamically perturb the enzyme molecule [15, 100]. The structure of the enzyme, particularly its size, may affect how it is influenced by sonication. The activity appears to be positively influenced by sonication in some enzymes [13], but sonication also damages enzymes especially if the temperature is not controlled. The free radicals generated by sonication and the shear forces associated with microstreaming may also affect the enzyme stability.

Sonication has been shown to improve a wide range of enzymatic processes [13, 101-103] and can be used to enhance bioethanol yield from lignocellulosic biomass [104]. Enzymes contribute significantly to the cost of producing lignocellulosic ethanol and, therefore, must be used effectively [104, 105]. Use of sonication can reduce processing costs by greatly reducing the amount of cellulase needed. In one case, the cellulase requirement was reduced to 50% [106]. Sonication facilitates enzyme action primarily by inducing flow close to solid–liquid interfaces to improve diffusion of the enzyme to the biomass [107]. Sonication generally improves mass transfer and mixing. Sonication may facilitate the binding of the

enzyme to the biomass substrate [37] and assist in removing the products of hydrolysis from the active site after the reaction is completed [107]. Low frequency sonication (<50 kHz) is preferred for use in enzymatic hydrolysis processes [108]. Low intensity sonication has accelerated enzymatic hydrolysis of corn stover and sugarcane bagasse [109].

In a study of enzymatic hydrolysis of sugarcane bagasse, separately grown cells of *Cellulomonas flavigena* were added to the slurry prior to sonication. Sonication disrupted cells to release the hydrolytic enzymes in the medium. These enzymes improved sugar yield but were eventually inactivated by prolonged sonication [90]. Sugarcane bagasse suspended in a solvent system of N-methylmorpholine-N-oxide (NMMO) and water has been enzymatically hydrolyzed using cellulase in an ultrasound assisted process [50]. Enzymatic hydrolysis at 50 °C could effectively depolymerize cellulose (~90% conversion) to glucose within about 12 h. In the same solvent system the sonication treatment at 90 °C in the absence of the enzyme converted <40% of the cellulose in 24 h [50]. Continuous sonication has been claimed to improve enzymatic hydrolysis more than intermittent sonication, supposedly because sonication facilitates both the binding and unbinding of the enzyme and the substrate [110, 111]. In simultaneous sonicated pretreatment and hydrolysis of lignocellulosic biomass, the lignin released can interfere with the action of cellulase [111]. In enzymatic hydrolysis of switchgrass biomass, sonication was reported to enhance sugar yield and the rate of hydrolysis compared to the use of enzyme alone [107]. Use of sonication has been reported to enhance the activity of cellulase by 70% in comparison to control [37].

5. 3. Fermentation

Although certain microbial cells can be extremely robust, live cells are readily disrupted by treatment with power ultrasound [13, 15, 23]. Sonication is, therefore, used to release intracellular proteins, organelles and other products from microbial cells [14, 15, 23, 45, 112, 113]. A fermentation process depends on the action of live cells. Therefore, overzealous sonication of a fermentation is counterproductive, but carefully tailored sonication regimens have been shown to actually improve the fermentation performance of live cells. Uninterrupted sonication has generally inactivated or killed microorganisms [114-115].

A growth accelerating effect of low-level sonication on the commonly used ethanol fermentation yeast *Saccharomyces cerevisiae* has been reported [116]. The duration of *S. cerevisiae* fermentation could be halved by applying low frequency sonication [117]. Using intermittently applied high-intensity sonication, Sulaiman et al. [21] reported improved growth of the yeast *Kluyveromyces marxianus* on dissolved nutrients. The productivity of ethanol from lactose was improved [21]. During simultaneous saccharification and fermentation of mixed waste paper slurry, ultrasound applied intermittently was found to stimulate the fermentation [118]. The final bioethanol concentration of ~37 g/L was 20% greater than in the non-sonicated fermentation [118]. Continuous application of ultrasound had a bacteriostatic effect and this reduced the ethanol yield [118].

Compared to enzymatic processes, processes involving live cells appear to be more sensitive to the intensity of sonication and whether sonication is intermittent or continuous. Sonication is not helpful in every microbial fermentation [119-120] and its efficacy needs to be established experimentally for each intended application. In certain processes, sonication regimes may be tailored to declump live microbial cells without damaging them [119-120].

6. Sonoreactors

Ultrasound assisted operations require the use of sonochemical reactors, or sonoreactors. The cavitational intensity declines exponentially with distance from the tip of the sonic horn

[71-72, 121]; thus, the efficacy of sonication in a liquid drops rapidly with distance from the source of the sound. As a result, sonication of a large volume of slurry using a point source may not be of beneficial [104, 111]. Effective sonication of a large volume requires specifically designed sonoreactors [13, 60].

Cavitational effects of sonication produce hotspots of high temperature and pressure within the fluid in a sonoreactor [22, 71]. In reaction systems that benefit from high-intensity sonication, these hotspots of intense activity determine the performance of the sonoreactor. Therefore, an accurate mapping of these hotspots is necessary for evaluating the performance of a sonoreactor and for its design [122]. Large zones of high cavitational activity may be desirable in a reactor for pretreatment of a lignocellulosic biomass. The mixing caused by sonication-induced microstreaming may be sufficient in a small reaction vessel, but supplemental stirring may be required in larger reactors to ensure that the fluid is repeatedly passed through the high-intensity cavitational zones [14]. Ultrasound induced mixing is affected by the location of the sonic transducer and the geometry of the vessel [123-124], but in a relatively large vessel, the mixing effect of sonication on a power per unit volume basis is much less than the effect of stirrer-induced mixing [123].

Conventional stirred tank reactors in conjunction with sonication have been effectively used for enzymatic saccharification of waste paper [111]. Other methods have been proposed for achieving uniform sonication in a reactor [125-127]. For example, multiple ultrasound transducers located at various positions of a vessel can be used to provide uniform cavitation [59, 71, 72, 128]. The scale up of a sonication treatment process is a considerable challenge [12, 62, 71, 72, 111], but is certainly not an insurmountable problem [13, 21, 60]. Although the conventional approach to scale up of a reactor vessel by simply increasing the size is not feasible, continuous flow sonication processes (Fig. 3) making use of a small sonicated volume, for example, may be an alternative [129]. The geometric configurations of sonochemical reactors can be as diverse as those of the conventional chemical and biochemical reactors [13, 58, 60, 71-72, 121, 130-133]. The factors affecting the design and performance of sonoreactors are further discussed in the literature [13, 14, 16, 58-60, 71-72, 131, 133-135].

Ultrasound horns, or transducers, and other equipment developed for sonic processing of chemicals may prove useful in bioprocessing. Sonic horns have been developed to improve the acoustic energy transmission and distribution into a fluid [136]. The diameter of the sonic horn determines the volume that can be effectively processed with it. A smaller tip generally provides a higher intensity sonication zone compared to a larger tip. Horns are commonly made of titanium, but the tip that is often designed to be replaceable, is made of a hard alloy or ceramic. Horn type of sonicators are suitable for small volumes [59], but may be used in a continuous flow operation for larger volumes. The tip of the horn is susceptible to erosion and pitting (Fig. 4) as a consequence of the cavitational phenomena that occur at the tip. Large sonoreactors generally require the use of multiple sonic horns, or other transducers [13]. Multiple horns allow the use of multifrequency operation [60]. The efficiency of sonication can be enhanced by positioning the transducers for positive interference within a sonoreactor. Multiple low intensity transducers minimize the risks of acoustic decoupling and erosion [59, 71].

7. Energy requirements of sonication

Sonication is an energy intensive process. Thus, commercialization of this process requires a through techno-economic analysis. Therefore, a sonication regime needs to be

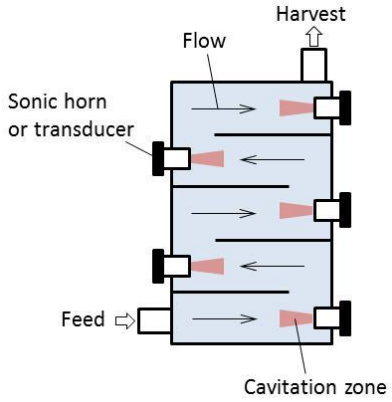

Fig. 3. A continuous flow ultrasonic reactor with multiple horns. Adapted from Nickel and Neis [138].

(a (b

Fig. 4. Erosion and pitting at sonotrode tip: new tip (a) and pitted tip (b). Courtesy of Qsonica LLC, Newtown, CT, USA.

optimized so that any unnecessary treatment is avoided. The overall processing cost, including the energy demand, is the principal factor that determines whether sonication is worthwhile compared to an alternative treatment. Sonication energy input depends primarily on the sonication power, the duration of sonication and the volume of the suspension being treated. The characteristics of the biomass will of course influence the nature of the treatment required [45]. The operating pressure also influences sonication as the sonication efficiency generally declines with increasing hydrostatic pressure.

The fraction of the supplied electrical energy that is actually dissipated in the fluid being sonicated, is the energy efficiency of sonication. All the sonic energy imparted to the fluid eventually reappears as heat, and can be measured by careful calorimetry. Within limits, the cavitational activity increases linearly with increasing energy input, but the design of the sonoreactor and the frequency of ultrasound affect this relationship. The energy efficiency of a sonochemical process increases with increasing energy input until the increasing number of cavitational bubbles formed in the vicinity of the sonotrode start interfering with each other. Formation of such bubble clouds decreases energy transfer to the fluid [60]. Power dissipation rate depends also on the surface area of the sonotrode and the properties of the fluid being irradiated. On an equal ethanol yield basis, a sonication pretreatment of lignocellulosic biomass can actually reduce the energy consumption by ≥50% relative to a nonsonicated equivalent pretreatment [74]. Therefore, there is a distinct potential for sonication being integrated in processes for producing bioethanol from lignocellulosic biomass.

8. Future prospects

The use of sonication in various stages of producing bioethanol from lignocellulosic biomass is relatively new. Nevertheless, studies in the laboratory have proved the technical

feasibility of sonication for biomass pretreatment, hydrolysis and fermentation. Efficacy of this technology needs to be further assessed for different types of lignocellulosics. The optimal processing conditions need to be identified [74] both for pretreatment, possibly in combination with inorganic chemicals and enzymes, and also for fermentation processes. Process economics and scalability need to be rigorously assessed [12, 45, 60, 62].

9. Concluding remarks

Ultrasound can be used to enhance the efficacy of some conventional methods of pretreatment of lignocellulosic biomass [139-141]. Sonication can improve also the conversion of the pretreated biomass to fermentable sugars and the fermentation of the sugars to bioethanol. In some cases at least, the improved performance achieved as a consequence of sonication appears to outweigh the added expense of sonication. The power consumption of some sonicated pretreatments can be actually lower per unit of ethanol produced, than the power consumption of the equivalent nonsonicated treatments. Pilot-scale studies are required to validate the promising results of the laboratory sonicated experiments and demonstrate a scale up capability for sonicated processing of lignocellulosic materials. Much work remains to be done on sonicated pilot-scale fermentations.

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