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# Intensification of Enzymatic Hydrolysis of Lignocellulose Using Ultrasound for Efficient Bioethanol Production: A Review

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**ABSTRACT:** Improvements in enzymatic hydrolysis for production of bioethanol from sustainable biomass are necessary in order to reduce enzyme requirements and the overall processing times. Conventional techniques for pretreatment of lignocelluloses are quite costly, time-consuming, and also require substantial downstream processing. Ultrasound can be effectively used to improve the hydrolysis process by reducing the structural rigidity of lignocellulose and by eliminating the mass-transfer resistances, which can contribute to increase in the product yield with reduced processing time and enzyme consumption. The present work initially presents an overview of different pretreatment methods involved in the enzymatic hydrolysis of lignocellulosic biomass highlighting its limitations. Expected mechanism of intensification due to the use of ultrasound has been discussed, also giving recommendations for optimum operating conditions. An overview of the different studies related to enzymatic hydrolysis using ultrasound for bioethanol production has been presented, along with discussion related to types of sonochemical reactors and scale-up aspects. Possible combination of sonication by coupling with traditional pretreatment processes has also been discussed. Overall, it appears that ultrasonic irradiations can be effectively used for the intensification of the enzymatic hydrolysis process for efficient bioethanol production.

## 1. INTRODUCTION

Declining global fossil fuel productive capacity, combined with a growing crude oil demand in developing countries, provides a compelling reason for alternative fuels. Biofuels have become more competitive, because of the existing stress on petroleum supply. Maintaining the current oil production levels requires the use of expensive technologies, because of the decreasing oil reserves, resulting in an increase in the final product prices. Also, increased demand further results in a considerable rise in oil prices. The change in global climate is also an additional important factor for the development of fuels with a reduced "carbon footprint". Therefore, the exhaustion of fossil fuel reserves and the stable increase in energy expenditure, oil prices, and environmental pollution has reawakened the interest to build up alternative energy sources, which could be renewable and environmentally friendly. Biofuels have the potential double benefits of global supply as well as reduced net life cycle greenhouse gas emissions, compared to their petroleum-derived alternatives. Ethanol produced using a renewable biomass is called bioethanol. It can be blended with gasoline or used as neat alcohol, exploiting the advantages of higher octane number and higher heat of vaporization. It is also an outstanding fuel for future advanced flex fuel hybrid vehicles.<sup>1</sup> Bioethanol also improves the fuel combustion in engines, thus reducing the emissions of carbon monoxide, unburned hydrocarbons, and other carcinogens. In comparison to gasoline, ethanol contains only a trace amount of sulfur. Therefore, ethanol blended with gasoline helps to decrease the overall sulfur content. This also lowers the emissions of sulfur oxide, which is a major component of acid rain, and also a carcinogen. The overwhelming advantage of bioethanol for the environment is its potential to be carbon neutral on a lifecycle basis, meaning the carbon dioxide (CO<sub>2</sub>) emitted during its use is counterbalanced by the absorption from the atmosphere during its growth.

Bioethanol can be produced from sugars, starches, or lignocellulosic biomass. Feedstocks derived from sugar and starchy materials currently dominate at the industrial level but even though they are economically favorable, there has been considerable debate about their sustainability. Lignocellulosic biomass such as corn stover, sugar cane bagasse, wheat and rice straw, forestry and paper mill residues and municipal waste, etc. are abundant, domestic, and renewable, and they are recognized as a potential low-cost source that can be converted to bioethanol. Also, it does not compete with food crops, and it is less expensive as compared to the conventional agricultural feed stocks.<sup>2</sup> Conversion of lignocellulosic biomass into bioethanol consists of the following steps: (i) pretreatment of lignocellulose to reduce recalcitrance in the lignocellulose by removing hemicellulose and lignin; (ii) enzymatic hydrolysis of cellulose to fermentable sugars; (iii) microbial fermentation of the sugars to ethanol or other valuable metabolites, and (iv) distillation.<sup>3</sup> Each of these steps (more importantly, step 1) can be a rate-limiting step in the overall process of bioethanol synthesis. Thus, the present work first provides an analysis of the different pretreatment options and is then followed by a discussion on novel approaches based on the use of ultrasonic irradiations (ultrasound) for enzymatic hydrolysis. To get a broader insight into the requirement of the pretreatment process, discussions related to the basics of enzymatic hydrolysis have been presented at the very outset.

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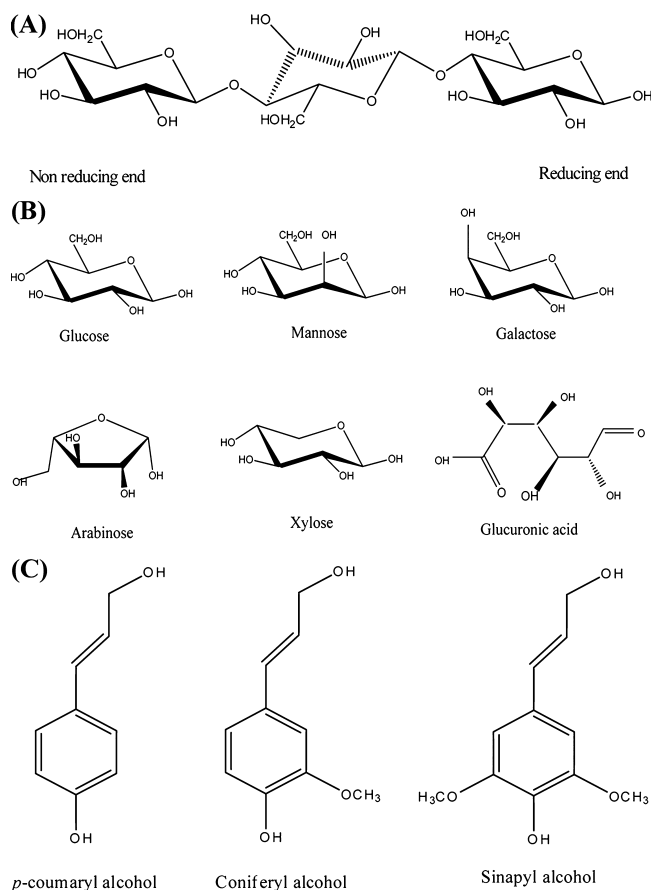
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## 2. ENZYMATIC HYDROLYSIS PROCESS

Lignocellulose is a component of plant cell walls composed of cellulose, hemicellulose, and lignin. The structure of cellulose, monomers of hemicellulose, and precursors of lignin are as shown in Figures 1A, 1B, and 1C, respectively. Cellulose is a



**Figure 1.** Structure of (A) cellulose, (B) monomers of hemicellulose, and (C) precursors of lignin.

linear high-molecular-weight polymer composed of glucose units connected by  $\beta$ (1-4) glycosidic linkages. Hydroxyl groups present in cellulose form intermolecular and intramolecular hydrogen bonds, which results in an ordered crystalline structure. Hemicellulose is a highly branched, heterogeneous polymer of various sugars, which is relatively amorphous. Lignin is a highly branched aromatic polymer, hydrophobic in nature and composed of propylphenol that binds to hemicellulose with covalent cross-linkages, embedding the cellulose fibers tightly.<sup>4</sup>

Enzymatic hydrolysis of cellulose is carried out by several catalytic enzymes. Three major groups of enzymes that are involved in the hydrolysis of cellulose are, namely, 1,4- $\beta$ -D-glucanoglucanohydrolase (EC 3.2.1.3), 1,4- $\beta$ -D-glucanocellobiohydrolase (EC 3.2.1.91), and  $\beta$ -D-glucosidase (EC 3.2.1.21). Based on the reaction they catalyze, these enzymes are generally termed as endoglucanase, exoglucanase, and cellobiase. The endoglucanase attacks randomly and hydrolyzes the cellulose chains to form glucose, cellobiose, and cellotriose. The nonreducing end of the cellulose is attacked by the exoglucanase to get the cellobiose units. Lastly, cellobiase converts cellobiose to D-glucose that can be fermented by yeasts or bacteria into ethanol.<sup>5</sup> Hydrolysis of cellulose in

lignocellulosic biomass can be carried out by using cellulase and particularly  $\beta$ -glucosidase to release the fermentable sugar.<sup>6</sup>

In addition to the three major groups of cellulase enzymes, there are also many subsidiary enzymes that attack hemicellulose, such as glucomannanase, glucuronidase, acetyl esterase,  $\beta$ -xylosidase, xylanase, and galactomannanase. The endoxylanase cleaves the main chains of xylan and  $\beta$ -xylosidase hydrolyzes xylooligosaccharides into xylose. The  $\alpha$ -arabinofuranosidase and  $\alpha$ -glucuronidase attacks the xylan backbone to remove the arabinose and 4-O-methyl glucuronic acid substituents, respectively.<sup>7</sup> Hemicellulolytic esterases are the acetyl esterases that attack the acetyl substitutions on xylose moieties, and feruloyl esterases attack the ester bond between the arabinose substitutions and ferulic acid. Feruloyl esterases assist the release of hemicellulose from lignin and render the opened polysaccharide product more acquiescent to degradation by the other hemicellulases.<sup>8</sup>

To obtain a higher sugar yield from lignocellulosic biomass, higher enzyme loading for hydrolysis can be used, but this ultimately results in an increase in cost. To avoid it, synergism between cellulase and others enzymes can be exploited. Hai et al.<sup>9</sup> have reported such synergism between cellulase and  $\beta$ -glucosidase for degradation of various types of celluloses.

## 3. FACTORS LIMITING ENZYMATIC HYDROLYSIS

The recalcitrance of lignocellulose is one of the major barriers to the economical production of bioethanol. Recalcitrance of lignocellulosic is known to be due to cell wall structure,<sup>10</sup> cellulose crystallinity,<sup>11</sup> degree of polymerization,<sup>12</sup> surface area,<sup>13</sup> and close association with lignin.<sup>14,15</sup> Similar to lignin, hemicelluloses also acts as a barrier in lignocellulosic biomass for enzymatic process by restricting access of enzyme to cellulose. Removal of hemicellulose increases the mean pore size of the substrate and therefore increases the probability of the cellulose hydrolysis.<sup>16</sup> Another limitation in using cellulase is that there is a reduction in rates due to end product (cellobiose and glucose) inhibition. The soluble inhibitors formed during lignocellulose processing also acts as a barrier. These inhibitors include soluble sugars, organic acids, furans, and phenolic compounds. These inhibitors causes cellulase deactivation, reducing both rate and yield of cellulose hydrolysis.<sup>17</sup> Various methods have been developed to reduce the inhibition caused by soluble sugars such as cellobiose and glucose, which includes using high concentration of enzyme, supplementation of  $\beta$ -glucosidase during hydrolysis or using simultaneous saccharification and fermentation (SSF) strategy. These methods resolve the inhibition problem by hydrolyzing cellulose and fermenting the hydrolysis product at the same time.<sup>18</sup> Among the polymeric phenols such as tannic acid, ferulic acid, and *p*-coumaric acid, tannic acid is the most damaging phenol, causing deactivation and a reversible loss of enzyme.<sup>19</sup>

## 4. PRETREATMENT TECHNOLOGIES

A pretreatment step is essential for achieving effective enzymatic digestion of lignocellulosic biomass for the production of bioethanol. Pretreatment alters biomass macroscopic and microscopic structure, as well as its chemical composition to facilitate efficient conversion of biomass to fermentable sugars. An efficient and economical pretreatment should fulfill the requirements such as production of reactive cellulose for enzymatic hydrolysis, avoiding the degradation of

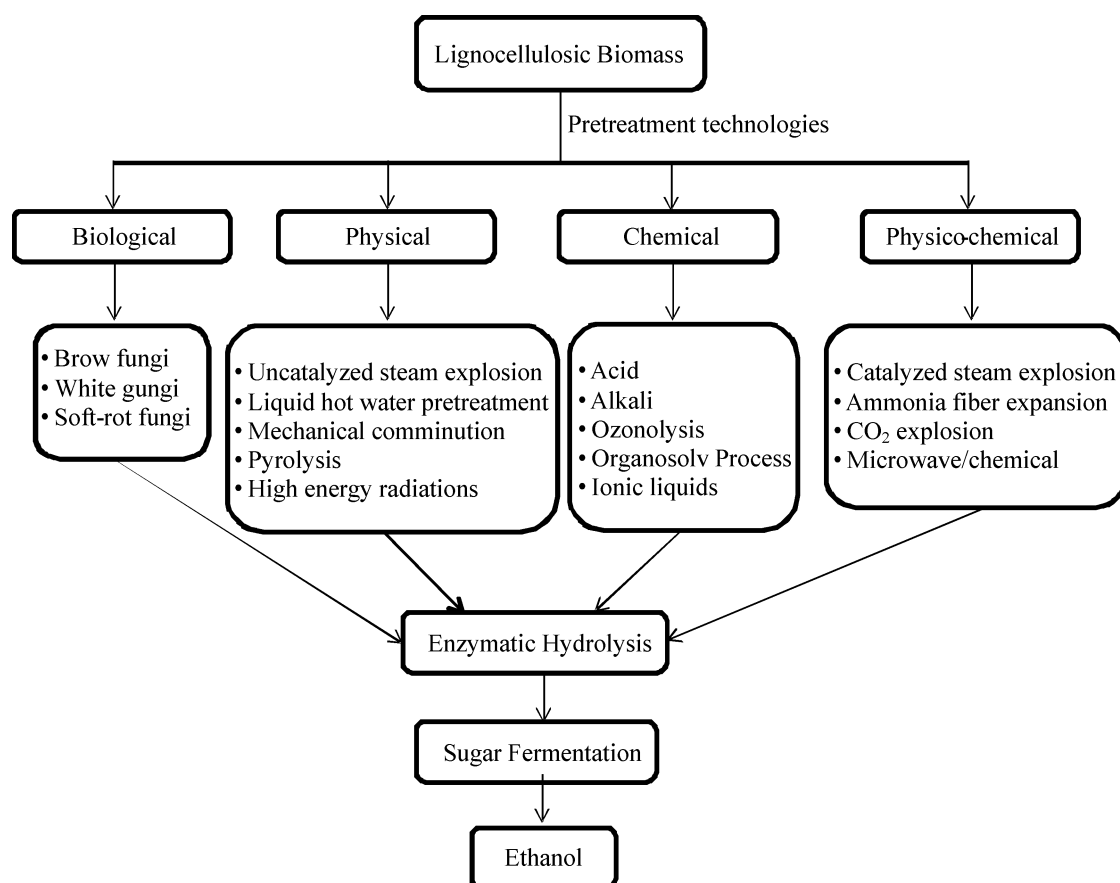


Figure 2. Schematic of pretreatment technologies used to produce bioethanol.

cellulose, minimizing heat and power requirement, and consumption of little or no chemical and allowing the use of economical materials. Various methods are available for pretreatment and are roughly classified into biological, physical, chemical, and physicochemical pretreatments. The schematic of pretreatment technologies to produce bioethanol is given in Figure 2. In this section, we review these methods, although it is important to note here that not all of them are developed enough to be feasible for applications in large-scale processes.

Biological pretreatment uses micro-organisms to degrade lignin. Biological pretreatment is a very safe and environmentally friendly method that does not require much energy. Micro-organisms such as brown, white, and soft rot fungi are generally used in biological pretreatment technique, in order to degrade lignin and hemicellulose in waste materials.<sup>20</sup> Low energy input, no chemical addition, and mild environmental conditions are the main advantages of biological pretreatment. However, the lignin degradation rate is very slow in most biological pretreatment processes and also the cost of downstream processing is very high.<sup>26</sup>

The different physical pretreatment technologies such as uncatalyzed steam explosion, liquid hot water (LHW), dilute acid, ammonia fiber expansion (AFEX), and lime pretreatment have been reviewed by Mosier et al.<sup>21</sup> and Wyman et al.<sup>22</sup> Uncatalyzed steam explosion is also termed as autohydrolysis, in which only high-pressure steam is used without the addition of any chemicals. In this method, biomass is rapidly heated by high-pressure saturated steam for a specific time to support the hemicellulose hydrolysis, and terminated by swift release of pressure, which causes explosive decompression. Although this

method can effectively improve the hydrolysis of the pretreated cellulose residue by enzymes, it suffers from low hemicellulose yield.<sup>23</sup> Therefore, it is still a problematic option for ethanol production on long-term basis. In liquid hot water pretreatment, high pressure is used in order to maintain water in the liquid state at high temperatures. This method is also termed as hydrothermolysis. Biomass undergoes high-temperature cooking in water under high pressure. LHW pretreatment has the ability to enhance cellulose digestibility, with the clear advantage of producing little or no inhibitor for the fermentation process.<sup>24</sup> Other pretreatments include catalytic pyrolysis and high-energy radiation and mechanical comminution. Pyrolysis has also been used for the pretreatment of lignocellulosic materials. When biomass is treated at temperatures more than 300 °C, cellulose rapidly decomposes to gaseous products and residual char. The presence of oxygen is known to aid the pyrolysis process.<sup>25,26</sup> Irradiation by  $\gamma$ -rays, electron beam, ultraviolet (UV) radiation, and microwaves can improve the digestibility of lignocelluloses. However, the irradiation methods are costly and have difficulties in industrial application.<sup>27,28</sup> Biomass materials can be comminuted by various milling, chipping, crushing, and grinding equipments. Mechanical comminution principally disrupts the crystallinity of cellulose, increases the specific surface area, and decreases the degree of polymerization. It breaks down the lignocellulosic biomass into smaller particles and thus exposes the substrate for consequent enzymatic hydrolysis. However, the mechanical comminution is time-consuming, and expensive; in addition, mechanical comminution is much less effective than chemical pretreatments since it does not result in lignin removal, which

restricts the accessibility of cellulose and inhibit cellulases. Therefore, it is infrequently used at present as a pretreatment method.<sup>29</sup>

Chemical pretreatments are purely initiated by chemical reactions for disruption of the biomass structure. Chemical pretreatments use acid, alkali, ozone, organic solvents, and ionic liquids (ILs). Acid pretreatment involves the use of concentrated or dilute acids to rupture the structure of the lignocellulosic biomass. At high temperatures, treatment of lignocellulosic biomass with acid can greatly improve the enzymatic hydrolysis. Sulfuric acid is the commonly used acid, which has been commercially used to pretreat a wide range of biomass like corn stover, switchgrass, etc., while other acids such as hydrochloric acid and nitric acid are less widely used.<sup>30</sup> Alkali pretreatment refers to the application of alkaline solutions such as sodium hydroxide (NaOH), calcium hydroxide (CaOH<sub>2</sub>), potassium hydroxide (KOH), or ammonia to degrade lignin, thus improving the reactivity of the cellulose. Alkaline hydrolysis is based on saponification of intermolecular ester bonds cross-linking xylan hemicelluloses and other components such as lignin.<sup>31</sup> Pretreatment of lignocellulosic materials, which is performed by treatment with ozone, is referred as "ozonolysis". Ozone has been reported to degrade lignin in many lignocellulosic biomasses, such as green hay, bagasse, pine, and peanut.<sup>32</sup> During ozonolysis, cellulose is not affected much, hemicellulose is slightly attacked, and mainly lignin is degraded. The major parameters in ozonolysis pretreatment are particle size of the biomass, moisture content, and ozone concentration in the gas flow. Organosolv processes use an organic solvent or mixture of solvents with water for removal of lignin from lignocellulosic feedstock. The organic solvents frequently used include ethanol, methanol, glycerol, acetone, aqueous phenol, ethylene glycol, tetrahydrofurfuryl alcohol, triethylene glycol, and aqueous *n*-butanol.<sup>33</sup> In the organosolv process, lignocelluloses are mixed with organic solvents and water and heated to dissolve the lignin and part of the hemicellulose, leaving cellulose ready for reaction in the solid phase. ILs are salts, characteristically composed of a small anion and a large organic cation, which have very low vapor pressure and remain as liquids at room temperature. ILs have recently received widespread attention on the cellulose dissolution. ILs shows promise as effective and "green" novel solvents, since they can dissolve large amounts of cellulose under considerably mild conditions and there is a possibility of recovering almost 100% of the used ILs to their original purity.<sup>34</sup> However, ILs are expensive and must be synthesized at lower cost and on a larger scale. The ability of micro-organisms to ferment sugars in the presence of these solvents also must be tested to carry out a continuous process.<sup>35</sup>

In physicochemical pretreatment, catalyzed steam explosion is similar to uncatalyzed steam explosion, except that acidic gases (and liquids), such as SO<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub>, CO<sub>2</sub>, and oxalic acid, are added as catalysts by impregnating the biomass before steam explosion. This method combines the effect of both acids and steam. Compared to uncatalyzed steam explosion, catalyzed steam explosion causes more-complete hemicellulose removal, leading to enhanced enzymatic digestibility of biomass. Destruction of a portion of xylan fraction and incomplete disruption of the lignin carbohydrate matrix are the limitations of catalyzed steam explosion.<sup>36,37</sup> Ammonia fiber expansion (AFEX) is theoretically similar to steam explosion in which biomass material is subjected to liquid anhydrous ammonia under high pressures and moderate temperatures

and is then rapidly depressurized. This quick reduction of pressure opens up the structure of lignocellulosic matrix, leading to increased digestibility of biomass.<sup>38</sup> CO<sub>2</sub> explosion method is similar to steam and ammonia fiber expansion with advantage of lower temperature than steam explosion and reduced expenses compared to ammonia expansion. In this method, high-pressure CO<sub>2</sub> is injected into the reactor and then liberated by an explosive decompression.<sup>39</sup>

However, the key factor that is limiting the use of various pretreatments is the cost of each of the pretreatments, even if high conversions are obtained subsequent to a cellulosic material being pretreated by any of these methods. An efficient pretreatment method is characterized by several criteria, such as preserving hemicellulose fractions, avoiding size reduction, preventing the formation of inhibitors by further degradation of sugar, minimizing energy requirement, and being cost-effective. Apart from these criteria, several other parameters, such as the recovery of high value-added co-products such as lignin, pretreatment catalyst, catalyst recycling, and waste treatment are also to be measured. For the evaluation of various pretreatment methods, all of the above-mentioned criteria should be considered.

Thus, there is clearly a need to develop an effective and economic alternative pretreatment technique to intensify the enzymatic hydrolysis of lignocellulosic biomass. We now highlight the use of sonication as an alternative and novel pretreatment approach, which also can be used to actually intensify the enzymatic hydrolysis process by simultaneous application at controlled intensity.

## 5. PROCESS INTENSIFICATION OF ENZYMATIC HYDROLYSIS USING SONICATION

**5.1. Introduction.** Process intensification is the development of novel reactors and techniques, in order to bring improvements in the processing, and considerably decrease energy utilization, or waste production. Process intensification is crucial for sustaining the chemical process industry.<sup>40</sup> Enzymatic bioprocessing of diverse substrates are usually specific to the target, and generates notably fewer effluents, which are also readily biodegradable, and do not create a significant environmental hazard. However, two shortcomings that obstruct its acceptance by the industry are costly processing and slow reaction rates.<sup>41</sup> The main objectives of various pretreatment systems are to increase the efficiency of the enzymes, and also to intensify the enzymatic process so that the overall process becomes economical. Cavitation is one of the effective process that can be used to intensify the enzymatic hydrolysis of lignocellulosic biomass. Cavitation is generally defined as the generation, consequent growth, and collapse of cavities, which, in turn, results in very high local energy densities.<sup>42</sup> When ultrasound (generally >20 kHz) passes through a liquid medium, the interaction between the ultrasound, dissolved gas, and liquid leads to an exciting phenomenon identified as acoustic cavitation. Conditions of very high temperatures and pressures (100–5000 atm of pressure and 500–15000 K of temperature) are generated locally for a period of microseconds by cavitation, while the overall environment remains equivalent to ambient atmospheric conditions.<sup>43</sup> Thus, cavitation allows the various physical processes and the chemical reactions to be conducted under ambient conditions.<sup>44</sup>

The mechanism of intensification of enzymatic hydrolysis process has been accredited to cavitation phenomena, which



results in the formation of intense turbulence and development of vibrations in the liquid system, which then leads to a significant increase in the mass-transfer rates. Ultrasound can be effectively used to increase the yield and rate of mass transfer in several enzymatic processes. In addition to these physical forces, the generation of microjets, shockwaves, shear, and acoustic streaming occurs, because of cavitation.<sup>45</sup>

We will now provide an in-depth analysis of different aspects related to intensification using an ultrasound-assisted enzymatic hydrolysis process. Different types of sonochemical reactors that can be used to intensify enzymatic hydrolysis processes have also been explained. The review highlights the combination of enzymatic hydrolysis and ultrasound, which can further intensify the process and result in economical operations, even at commercial scale operation.

**5.2. Mechanism of Sonication-Based Enzymatic Process Intensification.** The passage of ultrasound through a liquid medium results in the generation of cavitation events. Cavitation eliminates mass-transfer resistances in heterogeneous systems (Figure 3). Cavitation also produces a

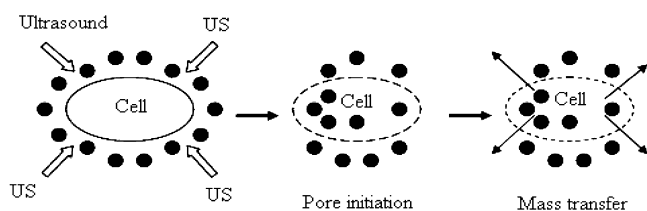


Figure 3. Enhancement of mass transfer using sonication.

hydrodynamic shear force in the aqueous phase due to the rapid collapse of microbubbles, which helps in the disintegration of coarser lignocellulosic materials into finer particles, thereby significantly increasing the surface area for enzymatic attack for bioethanol production, as shown in Figure 4.

High-energy intermediates, such as  $\text{H}\cdot$  (atomic hydrogen),  $\text{OH}\cdot$  (hydroxyl),  $\text{H}_2\text{O}_2$  (hydrogen peroxide),  $\text{HO}_2$  (superoxide) and  $\text{H}_2$  (molecular hydrogen) are produced by the collapse of cavitation bubbles in the liquid system.

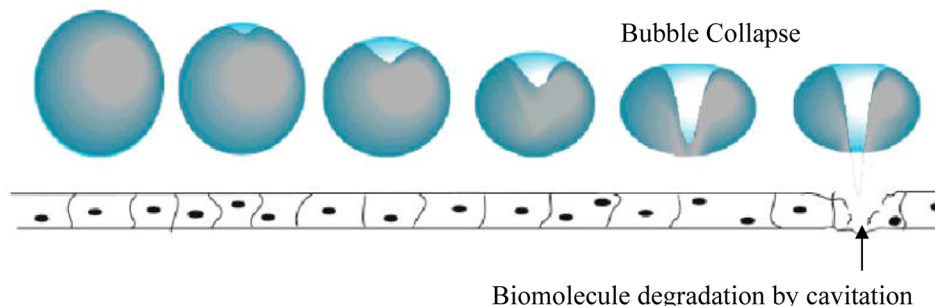
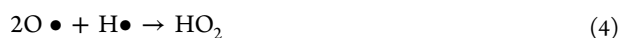


Figure 4. Mechanism of disintegration of biomolecule using sonication.

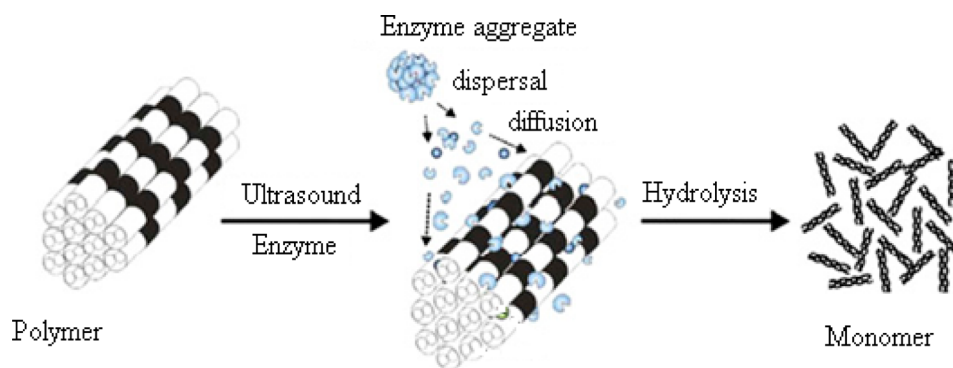
The formation of such highly reactive intermediates by collapsing cavitation bubbles should significantly affect the long-term catalytic stability and activity of the dissolved enzyme. The common insight was that these highly reactive intermediates could severely damage the very sensitive and complex enzyme structures. Conversely, when sonication was used specifically in an attempt to inactivate enzymes, its inactivation efficiency was moderately low.<sup>46</sup> One probable explanation for the evident inefficiency of ultrasound to inactivate enzyme macromolecules and cause enzyme substrate inhibition could be their extremely low ratio with solvent, since, typically, concentrations of 2–3 g/L are used for enzyme processing. Therefore, the possibility of enzyme macromolecules to be detained into a cavitation bubble and to come across the highly reactive intermediates created by the collapsing bubbles should be very low. Use of ultrasound under optimum conditions can enhance diffusion of enzymes to influence the rates of reaction. Also ultrasound decreases the barriers of transportation so that the enzyme can easily reach the target site, which then helps to increase the enzyme-to-substrate ratio at the target site and add to the reduction in reaction time.

The stabilization of enzyme in solution can also be improved by application of ultrasound because it prevents the enzyme agglomeration, as shown in Figure 5.<sup>47</sup> Ultrasound also influences enzymatic reactions in other ways, including probable effects on the formation and dissociation of enzyme–substrate complex. These effects ultimately lead to an increase in enzyme activity, as well as an increase in the overall rate of the enzymatic reactions.<sup>48</sup>

Ultrasound can also increase the yields of metabolites by improving mixing and phase transfer.<sup>49</sup> Application of ultrasound promotes cell membrane permeability and alters the surface potential resulting in activation of calcium channels, increases selectivity, accelerates nutrition transport, and enhances mass transfer inside and outside of the cell. Overall, the controlling mechanism of ultrasound-assisted enzymatic hydrolysis is generally attributed to mechanical and thermal effects, which results into a reduction in particle size and enhanced mass transfer across the cell membranes.<sup>50</sup>

## 6. OVERVIEW OF ENZYMATIC HYDROLYSIS PROCESS USING ULTRASOUND

Substantial recent effort has been spent in the use of ultrasound in combination with enzymes to try and maximize the quantity of glucose and, consequently, ethanol production from lignocellulosic biomass. Some mechanisms resulted in enhanced fermentation while others increase the amount of available cellulose from lignocellulosic materials. The effect of



**Figure 5.** Mechanism of prevention of enzyme agglomeration and depolymerization of lignocellulose using sonication.

ultrasound treatment on the saccharification of starchy materials has been widely studied. Table 1 provides a summary of effect of ultrasound on starchy materials. Important considerations have been also discussed in this section.

Effect of ultrasound pretreatment of sorghum slurry on the processes of liquefaction and saccharification was studied under optimized conditions.<sup>51</sup> The ultrasonic probe having a maximum power output of 750 W operating at a frequency of 20 kHz was used in the study. Because of ultrasound treatment, the liquefact dextrose equivalent (DE) increased by 10%–25%, depending on the sonication time and the intensity. The effects of high-power ultrasonics on the conversion of sugary-2 maize (*Zea mays* L.) to fermentable sugars were studied by Lomboy et al.<sup>52</sup> Ground sugary-2 maize mash was sonicated during hydrolysis at 20 kHz using three different amplitudes: low (164–174 W), medium (205–237 W), and high (248–330 W). It has been reported that the untreated control samples resulted in the lowest conversion at 13.7% while samples sonicated at low, medium, and high power for 40 s resulted in 39.2%, 48.8%, and 56.8% conversion, respectively. The effects of ultrasound and heat pretreatments on ethanol yields from cassava chips have also been investigated.<sup>53</sup> Cassava-starch-to-ethanol conversion efficiencies indicated that higher ethanol yields were directly related to sonication times during saccharification. Significantly higher ethanol yields were observed only for sonicated samples at the high-power level. The ethanol yield from the sonicated sample was 2.7-fold higher than the yield from the control sample. An ultrasound-assisted liquefaction as a pretreatment for bioethanol production by simultaneous saccharification and fermentation (SSF) of corn meal was studied by Nikolic et al.<sup>54</sup> Ultrasound pretreatment was performed at different sonication temperatures before the addition of liquefying enzymes. With an increase in the temperature, it has been reported that glucose concentration after liquefaction increases; however, at 80 °C, it decreased. Under optimal conditions, a maximum glucose concentration of 105 g/L was obtained after the liquefaction step. Ultrasonic energy can enhance liquefaction and saccharification of corn slurry both by pretreatment and simultaneous operation with enzymes. Enzyme addition during sonication gave higher glucose yields than enzyme addition after ultrasound (20 kHz) treatment. In addition, it has been reported that the glucose release was 30% higher when subjected to sonication, probably because of a decrease in particle size, better mixing due to streaming effects, and release of additional lipid-bound starch.<sup>55</sup>

Substantial recent effort has been spent in the use of ultrasound in combination with enzymes to try and maximize

the quantity of glucose and, consequently, ethanol production from lignocellulosic biomass. Some mechanisms result in enhanced fermentation while others increase the amount of available cellulose from lignocellulosic materials. The production of bioethanol from lignocellulosic materials is currently impeded by the high cost and low efficiency of enzymatic hydrolysis and cellulase activity. However, this can be partly conquered by the introduction of low-energy ultrasound. It has been established that enzyme efficiency greatly improves during the enzymatic hydrolysis of corn stover and sugar cane bagasse by using a 50 kHz ultrasound hexagon reactor system operated at 50 °C.<sup>56</sup> Elevated enzymatic hydrolysis yields after ultrasonic pretreatment can be explained on the basis of the fact that mechanical impacts, which are produced by the collapse of cavitation bubbles, open up the surface of solid substrates for enzymatic actions. In addition, the optimum temperature for many enzymes is 50 °C, and the maximum effects of cavitation occur at the same temperature. Synergistic effects are thus observed. Another reason responsible for beneficial results is that due to the heterogeneous nature of the system, the intrinsic cavitation activity is enhanced.

Garcia et al.<sup>57</sup> studied the effectiveness of variety of lignocellulosic biomass fractionation processes based on the use of ultrasound such as ultrasound-assisted organosolv, alkaline, and autohydrolysis treatments. The fractionation efficacy was calculated under different sonication conditions. The results showed that the use of ultrasound improved the yield and selectivity toward the product and that the obtained lignin did not undergo considerable modifications in its physicochemical properties. The effect of sonication pretreatment of rice straw for ethanol production was studied by Kangsadan et al.<sup>58</sup> Ultrasonic treatment of 0.16 kW/L was used during enzymatic hydrolysis and fermentation process. The concentration of glucose after pretreatment of rice straw during hydrolysis increased appreciably by 57.7%, as compared to the untreated sample.

Sono-assisted alkaline pretreatment of sugar cane bagasse for the production of fermentable sugar was studied by Velmurugan and Muthukumar.<sup>59</sup> The influence of various operating parameters such as particle size of material, liquid-to-solid ratio (LSR), NaOH concentration, sonication time, and temperature on delignification and production of reducing sugar were studied. For a maximum sugar yield of 92.11%, optimum parameters obtained were LSR of 25 mL/g, particle size of 0.27 mm, NaOH concentration of 2.9% (w/v), pretreatment time of 47.4 min, and temperature of 70.2 °C. Rolz<sup>60</sup> reported that the sugar cane bagasse that had previously been pretreated with ultrasound (20 kHz; 40 °C), enhanced the

Table 1. Overview of Application of Ultrasound for Starch-Based Materials

sample	application	sonication	mechanism	benefit	energy density	ref
1	intensifying glucose production from grain sorghum	750 W, 20 kHz	dry milling by mechanical shock	decreased the average particle size of the slurry from 302 to 115 $\mu\text{m}$ , increased saccharification by about 8%.	enzyme loading-0.08% (v/w), $E = 4500 \text{ J/mL}$	Shewale et al. <sup>51</sup>
2	sonication pretreatment to sugary-2 corn to enhance sugar release	2.2 kW, 20 kHz	degradation of rigid, crystalline structure by mechanical shock	particle size reduction, increase surface area for enzyme activity, 3-fold increase in sugar conversion	enzyme loading-0.5% (v/w), $E = 192 \text{ J/mL}$	Lomboy et al. <sup>52</sup>
3	ultrasound improved ethanol fermentation from cassava chips	2.2 kW, 20 kHz	shear force facilitates the disintegration of coarse particles into finer particles, significant increase in the surface area for enzyme activity	significant reduction in fermentation time, i.e., 24 h from 60 h and 29% increase in ethanol yield	enzyme loading-0.5% (v/w), $E = 192 \text{ J/mL}$	Nitayavardhana et al. <sup>53</sup>
4	ultrasound-assisted production of bioethanol by simultaneous saccharification and fermentation of corn meal	600 W, 40 kHz	depolymerization of starch granules	accelerate the starch hydrolysis to release glucose for further fermentation, 11.15% increase in ethanol production	enzyme loading-0.156% (v/w), $E = 360 \text{ J/mL}$	Nikolic et al. <sup>54</sup>
5	ultrasound-enhanced glucose release from corn in ethanol plants	20 kHz	sonication resulted in decrease in particle size and better mixing due to streaming effect	glucose release was 30% higher than that of control	Enzyme loading-0.05% (v/v), $E = 313.14 \text{ J/mL}$	Khanal et al. <sup>55</sup>

rate and extent of enzymatic saccharification by over 200%. It was suggested that due to acoustic streaming and local microturbulence, ultrasound influenced the dynamic adsorption-desorption enzyme mechanism.

The hydrolysis of cellulose from lignocellulosic biomass with just an enzymatic process is rather slow, but to improve the process, continuous irradiation of the ultrasound can be used.<sup>61</sup> Continuous application of ultrasound has been claimed to improve enzymatic hydrolysis more than intermittent sonication, because of the effective interaction between the enzyme and the substrate, which is based on the reality that sonication facilitates both binding and unbinding of the enzyme and the substrate. It has been conclusively recognized that after the significant delignification caused by loosening of the network, ultrasound (20 kHz; 45 °C) enhanced the saccharification by allowing access of enzyme toward the substrate. Ingram and Wood<sup>62</sup> presents a method for simultaneous saccharification and fermentation (SSF) using ultrasound to enhance the cellulase activity to hydrolyze mixed office waste paper, thus reducing the cellulose requirement such that the cost effectiveness of the process is enhanced. Table 2 provides the summary of application of ultrasound for lignocelluloses.

## 7. GUIDELINES FOR OPTIMUM OPERATING CONDITIONS

After the preceding discussion on the different methods for ultrasound-assisted enzymatic hydrolysis, the effectiveness and excellent benefits of the ultrasonic system have clearly been established. It is worthwhile to make some recommendations for the optimum operating parameters so that the overall operation becomes economically viable. The operating parameters that must be properly selected include the sonochemical reactor configuration, intensity of irradiation, operating frequency, sonication time in addition to the general parameters of enzymatic hydrolysis such as the operating temperature, enzyme load, enzyme-to-substrate ratio, etc.

**7.1. Immersion of Sonic Waves.** The sonochemical reactor configuration must be selected on the basis of ease of hydrolysis of lignocellulosic biomass. Ultrasonic horns belong to a type of direct immersion reactor that gives higher cavitation intensity as compared to ultrasonic bath, which are indirect irradiation-based sonochemical reactors. It would be essential to use reactors with multiple transducers for large-scale operation.<sup>63</sup> For direct-immersion-type reactors, it is suggested to construct a flow loop using multiple transducers immersed in the loop in contact with the flowing solution. For indirect-contact-type reactors, flow cells with transducers attached to the reactor walls or the bottom of the reactor can be constructed.

**7.2. Frequency of the Ultrasound.** At high ultrasound frequencies (on the order of megahertz, MHz), the production of cavitation bubbles becomes more difficult than at low ultrasound frequencies, of the order of kHz. In order to achieve cavitation, the intensity of the applied sound must be increased as the frequency increases, in order to conquer the cohesive forces of the liquid media and to create voids. The physical justification for this is based on the truth that the cycle of compression and decompression caused by the sound waves becomes so short at very high frequencies that the molecules of the liquid cannot be separated to form a void and, thus, cavitation is no longer achieved without the use of higher power dissipation levels.



Table 2. Summary of Application of Ultrasound for Lignocellulosic Biomass

sample	application	sonication	mechanism	benefit	energy density	reference
1	enhancement of enzymatic saccharification of kenaf powder by ultrasonic pretreatment	35 W, 24 kHz	ultrasound irradiation do cleavage of a $\alpha$ -O-4 or $\beta$ -O-4 linkages in lignin of kenaf powder	cellulose saccharification ratio increased by 86% after 15 min of the ultrasonic pretreatment	enzyme loading-6% (v/v), $E = 16800 \text{ J/mL}$	Ninomiya et al. <sup>70</sup>
2	utilization of sugar cane bagasse for bioethanol production using sono-assisted acid hydrolysis	400 W, 24 kHz	improves the transportation of substrates and enzymes through the cell wall	maximum glucose yield and amount get improved to 91.28% and 38.4 g/L respectively	$E = 1350 \text{ J/mL}$	Velmurugan and Muthukumar <sup>71</sup>
3	ultrasound assisted ethanol production during the simultaneous saccharification and fermentation of mixed waste office paper	36 kHz	cavitation disassembled cellulose fibers into component microfibrils increasing surface area available for enzymatic attack	ethanol production increased by ~20%	$E = 115.71 \text{ J/mL}$	Wood et al. <sup>72</sup>
4	Sono-assisted hydrolysis of sugar cane bagasse in cellulase- <i>aqueous-N-methylmorpholine-N-oxide</i> (NMMO) system for improved saccharification	100 W, 45 kHz	NMMO-treated cellulose became porous and amorphous	cellulose conversion rate increased by 51.2% and 57.8% compared to that of the untreated	enzyme loading-35 FPU cellulase and per gm of cellulose, $E = 288000 \text{ J/mL}$	Li et al. <sup>73</sup>
5	fermentation of rice hull under ultrasonic pretreatment	300 W, 28 kHz	surface erosion at liquid/solid interface or particle size reduction	enhance the saccharification process, accelerate enzymatic hydrolysis	$E = 6480 \text{ J/mL}$	Yang et al. <sup>82</sup>

In terms of the frequency of irradiation, a low frequency (such as 20 kHz) is recommended, which gives prevailing physical effects of cavitation in order to intensify the mass-transfer rates. If the operating frequency exceeds 100 kHz, the inactivation of the enzyme and microbial death may occur. A recent report on the enzymatic hydrolysis of cellulose using different ultrasonic frequencies shows that frequencies of 20 and 28 kHz are much more effective than 500 kHz.<sup>64</sup> By using a horn-type transducer at 20 kHz and the plate-type transducer at 28 kHz, the enzymatic hydrolysis process was accelerated by ultrasonic irradiation. Total sugar concentration produced linearly increased with ultrasonic intensity. Conversely, by using a plate-type transducer at 500 kHz, inhibition of enzymatic hydrolysis occurred and the total sugar concentration production decreased as the ultrasonic intensity increased. By using a horn-type transducer at 20 kHz, Khanal et al.<sup>55</sup> obtained a 30% increase in glucose production, relative to that of the control. It can be seen that simply increasing the frequency of irradiations to get enhanced results, especially for enzymatic processing, is not a good idea. Moreover, there exists an optimum frequency of operation, the extent of which usually depends on the specific system under study. Also, it is obvious that continuous operation with high-frequency irradiation leads to an erosion of the transducer surface. The power requirement for initiation of cavitation phenomenon in high-frequency operation is also much higher. Use of multiple frequency operation can be considered as a helpful substitute for the drawbacks related with the single frequency operation, particularly when higher cavitation intensities are required for the application.

**7.3. Intensity of Ultrasound.** The intensity of sonication is directly proportional to the amplitude of vibration of the ultrasonic source. Thus, an increase in the amplitude of vibration will lead to an increase in the sonochemical effects. Higher amplitudes are not always needed to obtain the desired effects especially in the case of enzymatic reactions. In addition, high amplitudes of sonication can lead to worsening of the ultrasonic transducer, which results in too much cavitation and poor transmission of the ultrasound through the liquid media. However, the amplitude should be increased for the liquid media with high viscosity. This is required because, as the viscosity increases, the resistance of the liquid medium for the movement increases. As a result, a high intensity is generally required to set the ultrasonic device to obtain the necessary mechanical vibrations in order to promote cavitation.

In terms of the power dissipation, usually optimum levels of power dissipation are recommended as higher power dissipation levels can result in deactivation of the enzyme. Exactly similar observations have been made in a study, when high ultrasound power of 500 W was used and ultrasound mediated deactivation of some enzymes was observed.<sup>65</sup> The observed results can be attributed to the reaction of hydroxyl or hydrogen radicals with the protein backbone. This consecutively could lead to aggregation of enzyme; thereby obstructing the active sites while simultaneously also decreasing protein stability. Moreover, shear forces that arise in the fluid because of ultrasound irradiation also have a considerable role in enzyme inactivation.

**7.4. Period of Sonication Treatment.** Another main parameter relating to economics of the enzymatic hydrolysis is sonication time. The sonication time required depends on the rigidity of the lignocellulosic material, the mass-transfer resistance for the diffusion of enzyme to the internal part of

the biomass, and the degradation rate of lignocelluloses. It is essential to describe the hydrolysis process in terms of kinetics, and then optimum treatment time for the ultrasound can be selected. It might be possible that the recovery of products either remains same or even decreases with the undue use of ultrasound. Studies for removal of hemicellulose and lignin from lignocellulosic biomass have shown similar results.<sup>66</sup>

Huang et al.<sup>67</sup> studied the effect of ultrasound on the structure and chemical reactivity of corn starch granules and reported that the degree of hydrolysis increased by a significant extent, when the time of sonication was increased from 3 min to 9 min, but continuing sonication beyond 9 min had a marginal effect on the degree of hydrolysis of corn starch. A less-recalcitrant starchy biomass requires short sonication duration, of the order of minutes, as compared to a more-recalcitrant lignocellulosic biomass. A sonicated alkaline pretreatment (15–35 min) increased the delignification of wheat straw by 8.4%, compared to nonsonicated sample. This enhancement was credited to the hydrodynamic shear forces related with ultrasound. In combination with alkali treatment, a lesser sonication treatment of 5–10 min did not attain significant delignification, compared with nonsonicated alkali pretreatment.<sup>68</sup> From the economic point of view, it is recommended to keep the sonication time short, since a large amount of energy is consumed by the application of ultrasound treatment. Generally, an ultrasound pretreatment of 5–10 min has been recognized to be suitable for enhancing the glucose yield in starchy materials, whereas for lignocellulosic material, a sonication time of 10–20 min would be proper.

**7.5. Temperature.** High solvent temperature has both advantages and disadvantages for ultrasonication. On one hand, disruption of the strong solute–matrix interactions like hydrogen bonding, van der Waals forces, and dipole attractions among the solute molecules and active sites on the biomass matrix occurs, and faster diffusion rates occur at high temperature. On the other hand, when the ultrasonic power of the system generator is constant, cavitation is better achieved at lower temperatures. This may be due to the fact that, as the temperature increases, vapor pressure of the solvent also increases and, therefore, more solvent vapor fills the cavitation bubbles, making the collapse less violent, and reducing sonication effects. Hence, an optimized temperature must be selected to obtain the utmost benefits.<sup>69</sup>

## 8. POSSIBLE COMBINED APPROACHES FOR PROCESS INTENSIFICATION

Enzymatic hydrolysis provides an environmentally friendly means of hydrolyzing cellulose with a probability of higher yield of glucose, but the costs required for the process are not favorable. Besides, cellulases are prone to strong product inhibition due to cellobiose and glucose. The combination approaches can be efficiently used with great advantages. To improve the release of fermentable sugar, the effect of ultrasound on the enzymatic hydrolysis of kenaf powder in ILs at room temperature has been reported by Ninomiya et al.<sup>70</sup> Five types of ILs have been studied for usefulness: 1-allyl-3-methylimidazolium chloride (AmimCl), 1-butyl-3-methylimidazolium chloride (BmimCl), 1-ethyl-3-methylimidazolium diethyl phosphate (EmimDep), 1-ethyl-3-methylimidazolium chloride (EmimCl), and 1-ethyl-3-methylimidazolium acetate (EmimOAc). Ground kenaf fiber mash was sonicated at varying amplitudes and a frequency of 24 kHz. By ultrasonic pretreatment, ~86% cellulose hydrolysis of kenaf powder in

EmimOAc was obtained in comparison with the only IL pretreatment.

Sono-assisted acid hydrolysis approach was used by Velmurugan and Muthukumar<sup>71</sup> for bioethanol production from sugar cane baggase (SCB). SCB was treated with 2% NaOH and ultrasound waves of 24 kHz for 20 min. Reduction in lignin content was observed to be ~75.4%, whereas cellulose and hemicellulose recovery was found to be 99% and 79%, respectively. For the stimulation of ethanol production during simultaneous saccharification and fermentation of office waste paper, Wood et al.<sup>72</sup> applied ultrasound. The availability of capillary-like regions, which increases the water-holding capacity of pulp was increased by ultrasonic treatment. Such changes in fiber structure may be favorable for the conversion of cellulose into ethanol. The effect of ultrasonic irradiations (36 kHz, 150 W for 15 min) on the stability and activity of  $\beta$ -glucosidase and cellulase was also been investigated and it was observed that  $\beta$ -glucosidase and cellulase activities remained stable over the period of 48 h and also ~50% reduction in the load of enzyme for the hydrolysis was reported.

Li et al.<sup>73</sup> studied the ultrasound-assisted hydrolysis of sugar cane bagasse in cellulase-aqueous-*N*-methylmorpholine-*N*-oxide (NMMO) system for enhanced saccharification. A system was established for effective saccharification of cellulose in NMMO-aqueous media in which the NMMO is capable to activate and solubilize the cellulose. After incubating into 15% and 20% (w/v) NMMO solutions, cellulase retained its original activity. High saccharification rate (96.5%) was obtained in aqueous-NMMO media by ultrasound assisted treatment of cellulose, after optimization of reaction parameters.

## 9. SONOCHEMICAL REACTORS AND SCALE-UP ASPECTS

The application of ultrasound requires a system capable of producing a stable and consistent ultrasonic field from electrical energy. The transducers are the devices used to convert the supplied energy, coming from a power generator, into mechanical energy in the form of vibrations. Mainly, two types of transducers exist: magnetostrictive and piezoelectric. The first one is usually constructed from high strength metallic alloys, which has the advantage of being able to reach high levels of acoustic power intensity, over 150 W/cm<sup>2</sup>. It is very stable, consistent, and does not deteriorate very easily.<sup>74</sup> However, it gives relatively low effectiveness (below 50%), if compared to piezoelectric systems (up to 95%). Piezoelectric transducers are more widely used, because of their higher transfer efficiencies, despite the relatively low levels of acoustic power intensity and the short life span. The transducers are attached to the vibrating system whose function consists of transmitting the vibration from the transducer to the liquid medium.

In case of liquid applications, the most frequently used systems are probe-type and bath-type systems. In the probe-type systems, ultrasound is directly applied by a vibrating horn. Horns, which consist of extenders, microtips, etc., are generally fabricated from high-grade titanium. The remainder of the reaction assembly with parts necessary to perform sonochemical reactions is generally fabricated with borosilicate glass. Ultrasonic horns are the most commonly used reactor designs among sonochemical reactors. These are the typically immersion type of transducers. Depending on the insertion and geometry of the probe, it could be used simply to transmit the ultrasonic energy or to amplify the intensity and, hence, the

net cavitation effects.<sup>75</sup> In the processes with this type of system, the distance between the horn tip and the treated sample is a significant factor to be controlled, because of the reduction in the ultrasonic field with the distance. Near to the horn, very high intensities are observed and the intensity decreases exponentially as one moves away from the horn.<sup>76</sup>

Peshkovsky<sup>77</sup> has developed "barbell-shaped" horns which can be used for the preliminary preparation with high amplitude processing, such as during pre-emulsification treatment of highly viscous fluids. These designs make use of high power ultrasound and produce immensely high ultrasonic amplitudes, although the resulting liquid processing capacity remains constant. Horst et al.<sup>78</sup> has developed the concentrator horn that operates at high intensity and produces longitudinal vibrations, which facilitates vigorous stirring of the working liquid.

Lomboy et al.<sup>79</sup> used a "donut shaped" ultrasonic horn that vibrated in radial directions. The horn was located in a vertical position inside the ultrasonication chamber, where almost all of the slurry passed through the center of the horn. Furthermore, even if the horn is radially vibrating, local measurements immediately below the horn also indicated high cavitation activity, which is yet again favorable in enhancing the global sonochemical yields. Similar types of radial vibrations are also obtained from a "Telsonic" horn, and this type of immersion system gives dual advantages of good distribution of the energy in the radial direction and greater irradiating surface.<sup>80</sup>

Ultrasonic baths are also commonly used, in which numerous transducers, vibrating in phase, can be attached underneath of a metallic tank transmitting the vibration to the liquid media. Because of the reflection of ultrasonic waves at the air/liquid interface, a stationary field consisting of maximum and minimum acoustic intensity zones is created. Therefore, in order to increase the maximum active zone inside the bath, the area of irradiating surface should be increased, to obtain better distribution/dissipation of energy in the reactor. This alteration helps to decrease the operating ultrasonic intensity, which increases the extent of the pressure pulse generated at the end of the cavitation events.<sup>81</sup>

Ultrasonic bath operating at 28 kHz and 300 W for pretreatment of rice hulls was used by Yang et al.<sup>82</sup> to improve sugar release for bioethanol production but nonuniform acoustic wave distribution was found in the reactor. Therefore, to facilitate the increase of active zones existing in the reactor and to avoid problems of nonuniform distribution of the cavitation activity, it has been recommended to change the position of the transducers, so that the wave patterns generated by the individual transducers will overlap. It also will result in uniform and increased cavitation activity. More recent developments have used direct bonding of the transducer to the surface of the vessel. Improvements in the bonding method have enabled the development of systems with large numbers of transducers to give an acoustic pattern, which is uniform and noncoherent above the cavitation threshold, throughout the working volume.

In order to have uniform distribution of sonic waves, Yachmenev et al.<sup>83</sup> used the advance sonic processing systems equipment with a dual frequency (16 and 20 kHz). This system produces a "beat" frequency that develops a highly energized zone oscillating continually and ensuring constant processing in the reaction chamber. Arrangements such as triangular pitch in the case of ultrasonic bath can also be used to avoid the problem of nonuniform distribution of sonic waves, but these

types of reactors are usually appropriate for small working volumes.<sup>84</sup> For continuous processing, tubular reactors with two ends irradiated with transducers or one end with transducer and other with a reflector can be used. Moreover, parallel-plate reactors with plates irradiated with either identical or different frequencies and hexagonal flow cells with transducers on each side of the hexagon can be constructed.<sup>85</sup> Dual frequency flow cell and triple-frequency flow-cell-type reactors have also been shown to be effective for large-scale continuous operation.<sup>86</sup> It is extremely important to have uniform distribution of the ultrasonic activity to increase cavitation effects. The use of low-output transducers gives the supplementary advantage of avoiding the phenomenon of cavitation blocking, which arises when power densities close to the delivery position are very high. In addition, these multitransducer units very efficiently concentrate ultrasonic intensity toward the central axis of the cylinder and away from the vessel walls.

There are many examples in the literature where the cavitation effects generated due to the application of ultrasound have been effectively used for physicochemical transformations but are limited to simple horn- and bath-type reactor configurations. The scaleup of horn-type systems is very poor, because it cannot efficiently pass on the acoustic energy into a large process volume. Also, these systems undergo corrosion and particle peeling at the delivery tip surface. The horn may also be subject to cavitation blocking (acoustic decoupling), and the large transducer dislocation increases strain on the material of construction, resulting in the possibility of breakdown. Also there is a lack of strong design and scale-up strategy establishing the significance of various aspects, such as hydrodynamics, mixing, and mass transfer that control the efficiency in the physical/chemical processing applications.

Transmission of the sound wave in the liquid results in the movement of fluid molecules. This movement described as acoustic streaming occurs in the direction of sound wave propagation, away from the transducer, because of the pressure gradient. The understanding of flow behavior is one of the main parameters in getting an effective scaleup of the reactor. This is because of the reality that the generation of acoustic streaming can achieve effective improvement in the rate of the transport processes specifically the heat and mass transfer in the case of multiphase reactions. Acoustic streaming currents also offer good mixing.<sup>87–89</sup> Figure 6 shows one of the reactor designs where cavitation can be utilized on a larger scale. This reactor has been used by Gondrexon et al.<sup>90</sup> for the degradation of an aqueous solution of pentachlorophenol in a three-stage continuous flow sonochemical reactor with an irradiating frequency of 500 kHz with a maximum power dissipation of 100 W. In this reactor system, cylindrical geometry was subdivided into three units, each interconnected by poly(vinyl chloride) (PVC) piping, which functions similar to a down-comer in the conventional distillation column.

Maintaining a consistent distribution of cavitation activity is one of the vital design aspects in the effective scaleup of the sonochemical reactors and can be obtained by optimizing the operating parameters (such as frequency, intensity, and power of irradiation) and geometric parameters (such as reactor configuration and number and location of the transducers).<sup>91</sup>



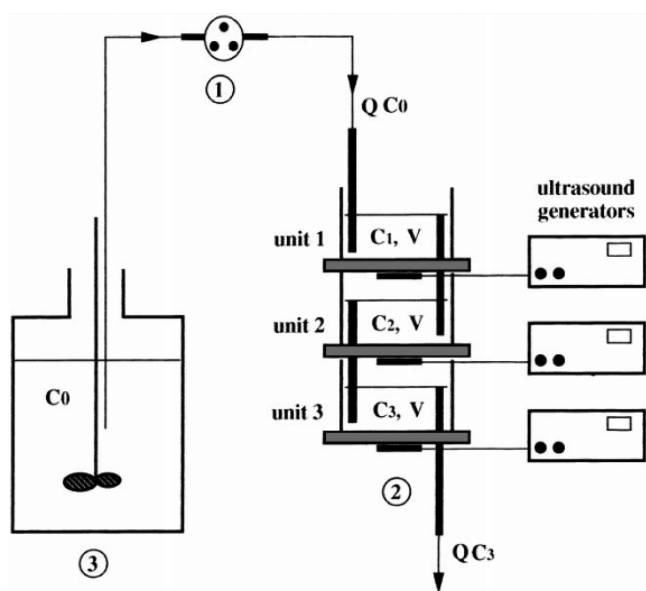


Figure 6. Schematic representation of sonication at a larger scale.

## 10. CONCLUSIONS

The discussion presented in the current review has clearly established the superiority of ultrasound-assisted pretreatment, compared to the conventional biological, physical, chemical, and physicochemical pretreatment processes for enzymatic hydrolysis for the production of ethanol from lignocellulosic materials. A major advantage of ultrasound-assisted pretreatment is the reduction of processing time for the same yield, in addition to other benefits, such as operation at lower temperature and a requirement of lower amounts of enzyme with minimum production of degradation products and inhibitors. The major interest from an industrial point of view is the higher yield obtained in this pretreatment process. Clearly, this technology is an “add-on” step to the existing process with minimum alteration, giving a reduction in enzyme usage, and a shortening of the treatment time. Ultrasound-assisted processes also can be coupled with other conventional techniques, possibly leading to a significant degree of process intensification. Based on current analysis, combining ultrasound with alkaline pretreatment seems to be a promising technique.

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### Notes

The authors declare no competing financial interest.

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