5aPA3. Enhancing biofuel production by ultrasonics

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This work evaluated the use of high-powered ultrasonics to enhance biofuel production in terms of efficiency and costs. A wide range of feed stocks, including corn, switchgrass, oleaginous yeast, and soybean oil were studied. The effect of ultrasonic pretreatment on the removal of lignin to allow hydrolysis of cellulose to fermentable sugars was studied. Many pretreatment techniques proved to be successful in enhancing lignin removal. For example, time of dissolution of ligno-cellulosic biomass in ionic liquids was reduced from hours to minutes, accompanied by a significant decrease in energy consumption compared to mechanical stirring. In addition, it was found that hydrolysis of corn starch could be greatly accelerated utilizing ultrasonics. Economic models showed that the technology, once implemented, would have a payback period of approximately 2.3 years. The work also explored biodiesel production using ultrasonics. It was seen that ultrasonics accelerated the transesterification process so that soybean oil could be converted to biodiesel in less than a minute, compared to 45 minutes using traditional methods.

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Introduction

The national interest in reducing energy dependence on foreign fossil fuels has led to a dramatic increase in the construction of biofuel plants in the United States. In more detail, dwindling reserves combined with the fact of rapidly increasing energy demands and the environmental issues caused by global warming, demand the development of sustainable, affordable, and environmentally friendly energy sources; they are all driving reasons for the development of efficient biomass to fuel technologies. Ethanol is a renewable, clean fuel currently produced from cornstarch in the US, and from sugarcane in Brazil. Another renewable fuel currently produced in the U.S. is biodiesel from soybean oil. As of October 2012, the annual ethanol and biodiesel produced were 10.09 [1] billion gallons and 781 million gallons [2], respectively. While ethanol from corn and biodiesel from soybean oil are currently the primary source of biofuels in the United States, a variety of second and third generation biofuels are studied and implemented, e.g., ethanol from a variety of lignocellulosic feedstocks and biodiesel from algal oil.

Ultrasound is sound waves at a frequency above the normal human hearing range (> 18-20 kHz). When the ultrasound wave propagates in a medium, such as in a liquid or slurry, it produces cavitation [3,4] and acoustic streaming [5]. The cavitation generates powerful hydro-mechanical shear forces in the bulk liquid [6], which cause disintegration of nearby particles. The main benefit of streaming in liquid processing is mixing, which facilitates the uniform distribution of ultrasound energy, convection of the liquid, and dissipation of any heating that may occur [7].

Ultrasonication has been applied widely in various biological and chemical processes. High-powered ultrasound has been used to enhance starch-protein separation in wet-milling operations [8]. Li et al. [9] utilized ultrasound to enhance oil extraction from soybeans, while Ebringerová et al. [10] used ultrasound to aid in the extraction of active xylan and heteroxylan from corn cobs and corn hulls, respectively. Wood et al. [11] studied the effects of ultrasonic treatment on ethanol fermentation from mixed office paper and demonstrated that sonication of recycled paper increased ethanol production by as much as 20%.

This paper is a summary of several, independent projects, all of which are detailed through the balance of this paper.

Ultrasonic Pretreatment of Corn Slurry

Because the release of starch from corn and the subsequent conversion of starch into fermentable sugar is an enzyme-mediated reaction, reducing corn particle size and efficient mass transfer of enzyme to substrate are critical to enhanced enzyme activity. However, ultrasound may also degrade enzymes because of localized heat, sonochemical reaction, and intense shear, if enzymes are added prior to sonication [12].

FIGURE 1. Effects of ultrasonication time on glucose release in commodity and sugary-2 corn. Zero ultrasonication time represents the control sample which was not exposed to ultrasonication.
Figure 1 shows the amount of glucose released after saccharification of corn slurry that was treated with ultrasonication. In this particular study, ultrasonic experiments were conducted using a Branson 2000 Series bench-scale ultrasonic unit (20kHz) at 320µm (peak-to-peak) amplitude for 0-40 seconds. Two types of corn were used, namely commodity corn and sugary-2 corn, which is also known as sweet corn. After sonication, enzymes (STARGEN 001, Genencor, Palo Alto, CA) were added, then the samples were saccharified for 3 hours. A control group was kept at the same condition without being exposed to ultrasonication. As seen in Figure 1, ultrasonicated samples showed a 2.2-10 fold improvement in sugar release compared to the untreated samples (control). These increases were observed in both commodity corn and sugary-2 corn. Also, both particle size distribution and scanning electron microscopy results (not shown here) confirmed that the corn particles were broken down to 1/20 of their original size, thereby increasing the surface area to volume ratio and thus dramatically increasing sugar release [7,13,14,15]. It should be noted that sugary-2 corn released more sugar than commodity corn because of its higher level of sucrose and amylose content [13]. Further experiments showed that, based on the energy calculation, for each Joule of ultrasonic energy introduced, up to 2 Joules of sugar equivalent energy was hydrolyzed from corn starch [7].

Economic Analysis of Ultrasonics and Jet Cooking

As with all technologies, acceptance depends on overcoming obstacles. For the biofuels, the efficiency of the selected process is one of the most critical challenges. For example, for any biofuel to become viable, energy input must not exceed the total energy output. There has been much debate on the net energy gain from converting cornstarch to ethanol [16,17]. Farrell et al. claimed that the net negative energy gain reported in the literature can be attributed to the neglect of co-products and to the use of some obsolete data in the calculation. There is also the possibility of improving the economics of dry corn milling ethanol plants by process improvements. For example, shortening liquefaction and fermentation times, lowering enzyme dosages [18], improving the overall starch hydrolysis, and eliminating some unit processes could cut production costs.

**Table 1.** Cost comparison model of jet cooking and ultrasonication as pretreatment. This model is based on Montalbo-Lomboy, et al. (2011) [15]. The economic analysis was done on 2009 dollars.

<table>
<thead>
<tr>
<th></th>
<th>Jet Cooker</th>
<th>Ultrasonics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Cost</td>
<td>85,955</td>
<td>806,450</td>
</tr>
<tr>
<td>Indirect Cost</td>
<td>27,207</td>
<td>255,265</td>
</tr>
<tr>
<td>Operating Cost ($C_{op}$)</td>
<td>869,475</td>
<td>296,471</td>
</tr>
<tr>
<td>Annual Capital Charges ($C_a$)</td>
<td>22,548</td>
<td>211,549</td>
</tr>
<tr>
<td><strong>Total Pretreatment Cost ($C_a + C_{op}$)</strong></td>
<td><strong>892,023</strong></td>
<td><strong>508,020</strong></td>
</tr>
</tbody>
</table>

Table 1 lists a cost comparison model of jet cooking and ultrasonication for corn slurry pretreatment [15]. The goal of the cost comparison model was to relate the current pretreatment technology (jet cooker) used for dry grind ethanol production to ultrasonic pretreatments as applied in our study. The system modeled in the analysis utilized a continuous ultrasonication approach, using a Branson Ultraso nics donut horn. The table shows that the overall costs for ultrasonic treatment were less compared to the use of a standard jet cooking system. For further details on the assumptions and the complete economic analysis, please refer to Montalbo-Lomboy, et al. (2011) [15].

Table 1 also shows that implementation of ultrasonics incurred higher direct and indirect costs than jet cooking; however, jet cooking incurred higher operating cost. If the capital funding was secured from a loan, only the capital charge will be paid annually instead of the total direct and indirect cost. The annual capital charge was calculated on a fixed annual interest rate of 15% over a payment period of 10 years. In this economic model, the annual pretreatment costs were the total costs of the annual operating cost ($C_{op}$) and the annual capital charge ($C_a$). Even though ultrasonics’ capital cost was nine-times higher than cost for the jet cooker, the total annual pretreatment costs for ultrasonics was still lower compared to the jet cooker. This is mainly caused by the high operating cost of a jet cooker.

To further evaluate the economic analysis, a net present value (NPV) and a benefit cost ratio (BCR) model were also developed. Both jet cooker and ultrasonics showed to be a viable and promising pretreatment method based on positive NPV values. However, the BCR value of ultrasonication was approximately 40% higher than the one for the jet cooker. Considering the possible savings from the total pretreatment costs when using ultrasonication instead
of jet cooking, ultrasonic pretreatment will obtain a return of investment period of approximately 2.3 years. This economic analysis shows that ultrasonics is a more cost effective investment than the jet cooker.

**Ultrasonic Treatment of Biomass with Autoclaving and Chemicals**

Currently, most ethanol in the U.S. is produced from fermentable sugars derived from the starch of corn kernels. However, considering that corn starch is a food resource, there is increasing concern about the economic and social impacts of redirecting a substantial portion of food resources into energy production.

Polyglucose is one of the main cell wall components in plants, such as switchgrass and other cellulosics materials. Although enzymes are currently available to convert these components into glucose (a fermentable sugar), the main challenge is to develop energy-efficient methods to separate fermentables, such as cellulose and hemicellulose, from non-fermentable inhibitory compounds, such as lignin. The lignin represents approximately 15% of most grasses (higher in woody plants) and is a major component of most agricultural residues. Lignin is a highly cross-linked polymer containing aromatic rings substituted by \( OCH_3 \), \( OH \), and \(-O-\) groups and a glycerol (three-carbon) alkyl chain. Although hemicelluloses are fermentable, they form tight complexes with lignin that makes them difficult to separate, thus protecting cellulose from enzymatic degradation. Lignocellulosic materials are mainly composed of celluloses, hemicelluloses and lignin. Because cellulose is locked in a three dimensional complex with hemicelluloses and lignin, it is difficult to extract. Lignin and its derivatives are known to be toxic to microorganisms and thus are inhibitory to enzymatic hydrolysis and fermentation. Therefore, it is important to separate lignin from cellulose prior to enzymatic hydrolysis and fermentation.

In a related study, our objective was to evaluate the effects of integrating ultrasound with physical and chemical pretreatment methods and to determine the best and most efficient ultrasonic conditions for removing lignin. Dry switchgrass (2.8 g) was weighed and mixed with either 1% sodium hydroxide or 1% sulfuric acid until the weight reached approximately 35 g. The sample mixtures were then sonicated at 96 \( \mu m_{ppp} \) (or low), 128 \( \mu m_{ppp} \) (or medium), and 160 \( \mu m_{ppp} \) (or high) amplitudes for three minutes. To ensure that the temperature did not increase significantly during sonication, the samples were immersed in an ice bath and temperatures were measured before and after sonication. The samples were then autoclaved at 121 °C for 1 hour. After autoclaving the samples were washed five times and dried for structural carbohydrate analysis. Figure 2 shows the lignin removed and glucan recovered after pretreatment.

As seen in Figure 2, the level of lignin removal was highest for the ultrasonically treated dilute sodium hydroxide samples. It is evident that dilute basic pretreatment exhibited better effects than dilute acid pretreatment in terms of lignin removal. However, it is interesting to note that there was only a very slight difference between the three ultrasonic amplitudes with regard to lignin removal. This indicates that a low ultrasonic amplitude was sufficient to effectively release lignin from the recalcitrant matrix of lignocellulosics.
FIGURE 2. Levels of lignin removal of ultrasonicated, autoclaved, and chemical treated switchgrass. Low U/S, med U/S, high U/S indicates sonication at 96 µm (peak-to-peak), 128 µm (p-p), and 160 µm (p-p) amplitudes, respectively. All samples with basic and acid labels were treated with 1% sodium hydroxide and 1% sulfuric acid, respectively. The NaOH and sulfuric acid only were samples not treated with ultrasonication and autoclaving.

Enhanced Biomass Dissolution of Ionic Liquid Using Ultrasonics

Another pretreatment method that has been attracting attention is the dissolution of cellulose in ionic liquid (IL). Several studies showed successful dissolution of lignocellulosic materials in ionic liquids [19,20,21]. Ionic liquids are advantageous compared to other solvents because they are chemically and thermally stable, non-flammable, and they have low vapor pressure [22]. In addition, it has been claimed that lignocellulosic dissolution in ionic liquids provides significantly higher enzymatic hydrolysis yields compared to other pretreatment methods, such as steam explosion and chemical treatment [23], producing high fermentable sugar yields within shorter times.

Despite the low vapor pressure of ionic liquids, studies have shown that ultrasonic energy enhanced the dissolution process in ionic liquids. In a study by Mikkola et al. [24], ultrasonic assisted cellulose dissolution occurred in 7-22 minutes, depending on the type of cellulosic material used. While it typically takes several hours to dissolve cellulose at room temperature using ionic liquids [25], with ultrasonication treatment, the dissolution time can be reduced by at least half the conventional room temperature dissolution.
FIGURE 3. Effects of ionic liquid dissolution on switchgrass using ultrasonication and heat treatment. Samples A and B were switchgrass after ionic liquid and ultrasonication treatment, while samples C and D were washed and dried switchgrass after ionic liquid dissolution. A – image of the sample at the beginning of ultrasonication, B – image of the sample during ultrasonication, C – scanning electron microscope image of the sample at 100x magnification after ionic liquid dissolution of switchgrass using heat treatment for 24 hours, D - scanning electron microscope image of the sample at 100x magnification after ionic liquid dissolution of switchgrass using ultrasonication for 3 minutes.

In our study, we explored the use of ultrasonication to enhance dissolution of switchgrass during exposure to an ionic liquid, methyl imidazolium chloride (BMIMCl). The objective was to compare the effects of ultrasonication to the ionic liquid dissolution process in comparison to conventional heat treatment. One gram of dried switchgrass was dissolved in 10 grams of BMIMCl, then sonicated at 160 µm (peak-to-peak) amplitude for 3 minutes. A similar sample was prepared and placed in a 130 °C oil bath. This sample was constantly stirred for 24 hours. As seen in Figure 3A and Figure 3B, the sample started out as clear (Figure 3A) then turned to black after ultrasonication. The same black colored sample was also observed for the heat treated samples after 24 hours. It is evident that ultrasonication has significantly reduced the treatment time from several hours to several minutes. The black color may be an indication that lignin has been solubilized.

After ultrasonication and heat treatment, 1:1 volume of acetone and water was added to the sample, which was then mixed for 1 hour. During mixing, the solubilized biomass precipitated. The solids were separated from the liquid by centrifugation. The solution was later washed several times to remove residual ionic liquid in the biomass, then freeze dried in preparation for enzymatic hydrolysis and structural carbohydrate analysis. Figure 3C and 3D show the scanning electron microscope images of the heat treated and ultrasonically treated biomass, respectively. They show that at the same magnification, the ultrasonically treated biomass contains smaller particles compared to heat treated samples. Further results (not shown here) showed that there was also a significant reduction in lignin in both heat treated and ultrasonically treated samples. The study concluded that ultrasonics can be an effective method to enhance the ionic liquid dissolution process of biomass.

Transesterification of Soybean Oil Using Ultrasound

Another renewable clean energy resource currently explored is biodiesel. Biodiesel is produced primarily from vegetable oil, but there are various other sources currently being used or studied for biodiesel production, such as animal fats, algae, fungi, and bacteria. Pure biodiesel sold as B100 can be directly used in standard diesel engines. Biodiesel is typically blended with standard diesel fuel. In more detail, B20 contains 80% diesel and 20% biodiesel. B5 blend contains 5% biodiesel and 95% diesel. Both of these blends are acceptable for use in most diesel engines. It is important that the biodiesel in these blends should be pure and meet the quality standard D 6751 specified by ASTM International (American Society of Testing and Materials). Biodiesel is a term given to fatty acid alkyl esters (FAME) produced by a transesterification reaction between a triglyceride and any alkyl alcohol. It is processed mainly from vegetable oils, is non-toxic, and free of sulfur and aromatic compounds. Transesterification is the process of modifying esters. Here, triglycerides, such as oils from vegetables, can be transesterified into long chain alkyl esters known as biodiesel. The reaction between a triglyceride (present in vegetable oil) and alkyl alcohol
produces alkyl esters and glycerol. Examples of triglycerides are vegetable oil, fatty tissues, or tallow obtained from animals. The alkyl alcohols generally used are methanol and ethanol. Figure 4 details the transesterification reaction between a vegetable oil, such as soy oil, corn oil, etc., and methanol.

![Chemical Structure]

**FIGURE 4.** Transesterification of triglycerides and methanol to form biodiesel and glycerin. The catalysts typically used are either sodium hydroxide or potassium hydroxide.

Transesterification reactions involve reactions between oil and alcohol in the presence of a catalyst. Oil and methyl alcohol are immiscible liquids and form separate layers when mixed together in a vessel. Traditional transesterification reaction requires continuous mixing for long periods of time to facilitate the reaction between oil and alcohol, because the reaction occurs only in the interfacial region between the two liquids. When this mixture is sonicated, ultrasonic waves produce cavitation in these interfacial areas. As a result, an emulsion of oil and alcohol forms, providing large surface areas for reaction, which in turn reduces reaction time significantly.

**FIGURE 5.** Biodiesel conversion from soybean oil using the commercial method, ultrasonic pulse method, and ultrasonic continuous method. The ultrasonic amplitude used was 120 µm (peak-to-peak). This result can also be found in Chand et al. (2010) [26].

In our studies with biodiesel, the objective was to compare the commercial heating method used for transesterification to ultrasonic assisted transesterification. A mixture of 600 ml soybean oil, 150 ml methanol, and 6 g of sodium hydroxide was prepared. For the commercial heating method, the mixture was allowed to react at 60 °C in a shaking water bath. Samples were taken from the reaction mixture at predetermined times. Biodiesel was analyzed using thermogravimetric analysis. For the ultrasonic assisted experiments, 10 ml of soybean oil was mixed with 2.5 ml methanol and 0.1 g sodium hydroxide. The sample size was scaled down because of the limited reactor size for the ultrasonic experiments. The mixture was sonicated using an ultrasonic pulse mode with a 5 s on and 25 s off cycle. The samples were collected at the end of every 30, 60, 90, 120 and 150 seconds. Three amplitude levels
were tested: 60 µm(p-p), 120 µm(p-p) and 180 µm(p-p). Another ultrasonic experiment was conducted during which the samples were continuously sonicated for 15 seconds at 120 µm(p-p).

As seen in Figure 5 [26], both the commercial method and ultrasonic assisted method achieved 100% biodiesel conversion. However, it is interesting to note that with the ultrasonic assisted method, full conversion was achieved in significantly reduced time compared to the commercial method. It is important to note that in this figure the data was plotted in a log/log plot. Based on this results, it is evident that ultrasonication offers a very promising potential to improve the transesterification process during biodiesel production.

**Summary**

Various studies investigated the utilization of ultrasonication in biological and chemical processes. In this study, ultrasonication was especially explored to improve production of biofuels, e.g., ethanol from corn and switchgrass, and biodiesel from soybean oil and oleaginous yeast. These studies showed that ultrasonication of corn slurry prior to saccharification improved the glucose yield by at least a factor of two in both commodity and sugary-2 corn. In addition, economic analysis indicated that ultrasonic treatment was more cost effective compared to jet cooking. This study also showed that ultrasonics combined with autoclaving and chemical treatment, e.g., with dilute acid or dilute base, can be an effective method to remove lignin from the recalcitrant lignocellulosics matrix. During ionic liquid dissolution of biomass, ultrasonics improved the process by reducing the dissolution time from several hours to several minutes. Ultrasonic assisted biodiesel production was also investigated in this study. The study found that ultrasonication significantly reduced the reaction time from 45 minutes (with commercial heating method) to 1.5 minutes (with ultrasonic pulse method), and 15 s (with ultrasonic continuous method) respectively. Overall, this study showed that ultrasonication provides potential to improve biofuel processes.

**ACKNOWLEDGMENTS**

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**REFERENCES**