Ultrasonic pretreatment for enhanced saccharification and fermentation of ethanol production from corn

by

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ABSTRACT

The 21^{st} Century human lifestyle has become heavily dependent on hydrocarbon inputs. Energy demand and the global warming effects due to the burning of fossil fuels have continued to increase. Rising awareness of the negative environmental and economic impacts of hydrocarbon dependence has led to a resurgence of interest in renewable energy sources such as ethanol. Fuel ethanol is known to be a cleaner and renewable source of energy relative to gasoline. Many studies have agreed that fuel ethanol has reduced greenhouse gas (GHG) emissions and has larger overall energy benefits compared to gasoline. Currently, the majority of the fuel ethanol in the United States is produced from corn using dry-grind milling process. The typical dry-grind ethanol plant incorporates jet cooking using steam to cook the corn slurry as pretreatment for saccharification; an energy intensive step. In aiming to reduce energy usage, this study evaluated the use of ultrasonics as an alternative to jet cooking.

Ultrasonic batch experiments were conducted using a Branson 2000 Series bench-scale ultrasonic unit operating at a frequency of 20 kHz and a maximum output of 2.2 kW. Corn slurry was sonicated at varying amplitudes from 192 to 320 $\mu m_{\text{peak-to-peak(p-p)}}$ for 0-40 seconds. Enzyme stability was investigated by adding enzyme (STARGEN$^{\text{TM}}$001) before and after sonication. Scanning electron micrograph (SEM) images and particle size distribution analysis showed a nearly 20-fold size reduction by disintegration of corn particles due to ultrasonication. The results also showed a 30% improvement in sugar release of sonicated samples relative to the control group (untreated). The efficiency exceeded 100% in terms of relative energy gain from the

additional sugar released due to ultrasonication compared to the ultrasonic energy applied. Interestingly, enzymatic activity was enhanced when sonicated at low and medium power. This result suggested that ultrasonic energy did not denature the enzymes during pretreatment.

Ultrasonication of sugary-2 corn was also investigated in the study. Results similar to those for commodity corn (dent corn) were found, in terms of glucose yield and starch conversion. SEM and polarized-light microscope pictures showed the partial gelatinization of corn slurry due to ultrasound. In the 96-h saccharification time, a model was formulated to fit the sugar release curve. The results have shown 17-21% increase in the extent of sugar production from sonicated samples relative to the control group. Additionally, the reaction rates of the sonicated samples were 2- to 10-fold higher than the reaction rates for the control group. In comparing sugary-2 corn with commodity corn, it was found that sonicated sugary-2 corn saccharified faster than sonicated commodity corn. It is important to note, without ultrasonic treatment, sugary-2 corn released more reducing sugar than commodity corn during saccharification.

To further investigate the potential of ultrasonics for scale-up, a continuous flow system was studied. An ultrasonic continuous flow system was tested using Branson's flow-through "donut" horn. The donut horn, which vibrates radially, was placed inside a 5.5 L stainless steel reactor. The amplitude was maintained at 12 μm_{pp} and the feed flow rate was varied from 8-27 L/min (2-7 gal/min) with reactor retention times varying from 12-40 seconds. Samples sonicated in continuous flow system showed lower reducing sugar yield than batch ultrasonication. However, considering the ultrasonic energy density of batch and continuous systems, the continuous systems proved to be more

energy efficient in terms of glucose production compared with the batch system. It was also seen that particle size disintegration was proportional to energy density regardless of the type of ultrasonic system used.

To compare ultrasonics with jet cooking, fermentation experiments were conducted. There were only marginal differences between jet cooked samples and the sonicated samples in terms of ethanol conversion based on theoretical yield. Furthermore, statistical analysis confirmed that there was no significant difference ($p<0.05$) in the ethanol yields of the two pretreatment methods. Economic analysis indicated that the capital cost of installing ultrasonics was higher compared to jet cooker equipment. However, due to the energy needs of jet cooking, a typical 189 million liters (50 million gallon) per year ethanol plant ethanol plant would save about 16% in pretreatment cost by using ultrasonics.

Based on these results, ultrasonication is a promising pretreatment method in corn ethanol production, as an alternative to jet cooking.

CHAPTER 1: INTRODUCTION AND OBJECTIVES

1.1 Introduction

“Our current living standard could not be maintained without energy” [1]. In 2007, the U.S. annual energy consumption reached 101.6 quadrillion Btu [2]. It was reported that 84.9% of the total energy consumed was obtained from fossil fuel [2]. The use of fossil fuels has been known to contribute to environmental pollution. This effect is especially pronounced in the emission of greenhouse gases in combustion of gasoline, as well as burning of coal. U.S. preliminary data showed 5890 MMT (million metric tons) of CO_2 emission in 2006, and 34% is attributed to the transportation sector [3]. Aside from the environmental impact, the cost of imported crude oil has also increased 10-fold in the last decade reaching $140 per barrel in June, 2008. This increased cost poses a great threat to the national security of the country. These pressing issues have compelled us to look into alternative fuels that are cleaner and renewable.

Since as early as 1920s, ethanol has been blended in gasoline as an octane booster [4]**.** Ethanol is a renewable and clean fuel [5]. When ethanol is added to gasoline, it enhances fuel combustion, reduces tailpipe emissions of carbon monoxide (CO), and reduces smog formations [6]. With advancements in biotechnology, ethanol production has been improved to such an extent that it has become a competitive industry. Additionally, with the tax incentives implementation of the Energy Policy Act (EPACT), more producers and farmers were also encouraged to grow feedstocks such as corn for fuel ethanol.

Currently, ethanol is produced from corn using either a wet or dry milling process. Due to the lower production cost of the dry milling process, more than 80% of the ethanol in the US is produced using this process. In dry milling, the whole corn kernel is ground into fine particle sizes and processed without separating the components of the kernel. The ground corn is mixed with water to form slurry that is then cooked using steam in hydro cookers. Enzymes are added to the corn slurry to convert the starches to glucose. The cooking step gelatinizes the starchy materials in corn and sterilizes the corn slurry in preparation for fermentation. The average thermal energy used in a typical dry-grind milling plant is more than 10MJ per liter of ethanol produced [7]. Assuming a fraction of this thermal energy is used for jet cooking, a conservative 5% reduction in thermal energy usage is possible when jet cooking is eliminated or replaced. With the aim of reducing the energy usage, this study proposes ultrasonic treatment as a potential pretreatment method that requires significantly less energy input than jet cooking.

Ultrasonics is sound waves at frequencies above the normal hearing range (> 18 kHz). Ultrasonic technology has been widely used in a variety of applications from low-power medical diagnostics to high-power bacterial disinfection. Several studies have shown that starch degradation can be induced by ultrasonics [8,9,10]. Additionally, Mason et al. [11] cited the positive effects of ultrasound on enzyme activity. For these reasons, ultrasonication exhibits potential benefits as an alternative method of corn slurry pretreatment.

1.2 Dissertation Objectives

The dissertation has five separate objectives, listed below. The overall aim is to determine the effect of ultrasound in corn slurry as a pretreatment method for ethanol production. This research includes investigation of saccharification and fermentation of corn slurry treated with ultrasonics. The detailed objectives of the dissertation and brief description of the approach to the research are as follows:

1. To investigate the effects of ultrasonics on particle size, glucose release, enzyme stability, and corn morphology at varying power intensities. This investigation was conducted on bench-scale in a batch reactor using commodity corn (yellow dent).
2. To determine the effects of ultrasound on sugary-2 corn as compared with commodity corn. The study assessed the effects of ultrasonics on both types of corn based on swelling power, water solubility index and saccharification yield.
3. To investigate the effects of lab-scale continuous flow ultrasonics setup as a potential scale-up saccharification pretreatment method. This investigation involved comparison of the saccharification yield and particle size characterization of the ultrasonic batch mode and continuous mode.
4. To study the effects of ultrasound on the fermentation yield of commodity corn slurry. This investigation also included comparison of the fermentation yield between corn slurry treated with ultrasonics and conventional jet cooking method.

5. To evaluate the economic aspect of applying ultrasonics in ethanol plants. The economic analysis involved a comparison of the cost of ultrasonication and conventional jet cooking as pretreatment methods in an ethanol plant.

1.3 Dissertation Organization

This dissertation is organized into seven chapters and an appendix. The Introduction and Objectives and the Literature Review are Chapters 1 and 2, respectively. The following four chapters consist of four manuscripts prepared for publication in various international journals. The first manuscript has been published in *Biotechnology and Bioengineering* in December 2007, while the second manuscript was submitted to *Bioresource Technology* in April 2008. The third and fourth manuscript will be submitted to *Biotechnology Progress* and *Ultrasonics Sonochemistry*, respectively. The general conclusions and recommendations for this dissertation are summarized in Chapter 7. Finally, the appendix tabulates all the experimental data presented in this dissertation. It is noted that references for chapters 1 and 2 are found at the end of chapter 2 while the literatures cited in chapters 3, 4, 5 and 6 are found at the end of each chapters.

Chapter 3, “Ultrasound Enhanced Glucose Release from Corn in Ethanol Plants,” evaluates the use of ultrasonics as pretreatment for corn slurry for enhanced liquefaction and saccharification. The paper provides an overview, which aims to satisfy the first objective of this dissertation.

Chapter 4, “Sonication of Sugary-2 Corn: A Potential Pretreatment to Enhance Sugar Release,” discusses the effects of ultrasound irradiation on sugary-2 corn. The method used in this paper were similar to that in the Chapter 3 manuscript but provides more fundamental details on starch conversion, starch structure, and gelatinization. The manuscript aims to satisfy objective 2 of this dissertation.

Chapter 5, “Characterization of Particle Size and Saccharification in Batch and Continuously Ultrasonicated Corn Slurry,” reports on studies of the effects of ultrasonics on corn slurry in both batch and continuous flow modes. The particle size characterization involved correlation of the particle size distribution to corn morphology. This manuscript aims to satisfy the third objective of this dissertation.

Chapter 6, “Ultrasonic Pretreatment of Corn for Simultaneous Saccharification and Fermentation in Ethanol Production” describes the effect of ultrasonic pretreatment on fermentation yield such as ethanol. The economic possibilities of applying ultrasonics in a large scale set-up are also discussed. Additionally, the paper compares ultrasonics with conventional jet cooking as presaccharification treatment. Objectives 4 & 5 will be satisfied in this manuscript.

CHAPTER 2: LITERATURE REVIEW

2.1 Energy and the Environment

To improve and maintain a comfortable lifestyle, civilization places great reliance on energy. In 2005, world annual energy consumption was 462.8 quadrillion Btu [12], where 26.3%, 32%, and 18.6% of this overall consumption account for North America, Asia, and Europe, respectively. According to the U.S. Energy Information Administration (EIA), based on the current trend, the world energy consumption is expected to increase to 694.7 quadrillion Btu in 2030 [12]. In the United States alone, the 2007 annual energy consumption was reported to be 101.6 quadrillion Btu [2]. Roughly 32.5 % of this overall energy consumption is ascribed to the industrial sector, 27.8% to the transportation sector, and 21.5% and 18.2%, respectively, to the residential and commercial sectors. The report also indicated that about 39% (~39.8 quadrillion Btu) of the energy used is derived from petroleum, more than 70% of which is imported. This is clearly an indication of the high dependence of the U.S. on foreign oil.

Looking closely at fossil fuel consumption, only petroleum is substantially imported from other countries, as there is an abundance of coal and natural gas in the U.S. [2]. However, due to the severe oil price increases in recent years, the United States and many other countries have tried reducing oil imports and developing new sources of energy. The transportation sector is the largest consumer of petroleum and has been the main area for alternative fuel development.

Beyond its large petroleum utilization, the transportation sector also generates a severe environmental impact that leads to climate-changing events. By definition, climate change is referred to as “any significant change in measures of climate (such as temperature, precipitation, or wind) lasting for an extended period” [13]. These changes may occur as a result of “natural factors such as slow changes in the Earth’s orbit around the sun, natural processes within the climate system (change in ocean circulation), or human activities that forcibly change the atmosphere’s composition (deforestation, urbanization and fossil fuel burning” [13]. The transportation sector has been a major contributor to carbon dioxide (CO_2) in the atmosphere, which eventually leads to global warming. Additionally, incomplete combustion also leads to production of toxic carbon monoxide (CO). Moreover, approximately 40% of the gaseous compounds released to the atmosphere by vehicular traffic also caused ozone formation and smog, especially prevalent in cities [6].

Since industrialization, the burning of fossil fuels has caused the accumulation of heat trapping “greenhouse gases” to considerably increase in the atmosphere [13]. Greenhouse gases such as carbon dioxide, methane, and nitrous oxide are necessary to life on this planet [13]. These gases prevent heat to escape to space, thus keeping the Earth warm [13]. The accumulation of these gases in the atmosphere leads to increase in the Earth’s temperature and eventually affect the planet’s climate [13].

Table 2.1 shows an excerpt of data for the U.S. carbon dioxide emissions of the transportation sector from 1990 to 2006 [3]. It can be seen that the preliminary carbon dioxide emissions from petroleum in 2006 were 409.2 million metric tons higher than 1990. EIA indicated that this increase “represents 46.4% of the growth in unadjusted energy-related carbon dioxide emissions from all sectors over the period” [3].

Additionally, NASA records confirm that Earth's top 5 warmest years (since 1880) occurred in the year 2005, 1998, 2002, 2003 & 2006 (arranged in descending order) [14].

Table 2.1 U.S. Carbon Dioxide Emissions from Transportation Sector [3] (Million Metric Tons CO_2)

	1990	**1995**	**2000**	**P2006**
Petroleum				
Motor Gasoline	961.7	1029.7	1121.9	1186.2
Liquid Petroleum Gasoline	1.3	1.0	0.7	1.1
Jet Fuel	222.6	222.1	253.8	239.5
Distillate Fuel	267.8	306.9	377.8	452.2
Residual Fuel	80.1	71.7	69.9	65.6
Lubricants	6.5	6.2	6.7	5.5
Aviation Gasoline	3.1	2.7	2.5	2.3
Petroleum Subtotal	**1543.2**	**1640.4**	**1812.5**	**1952.4**

Note: Totals may not equal sum of components to independent rounding.
P – preliminary data.

2.2 Renewable Energy

In recent years, renewable energy has gained more attention due to the demand for a sustainable energy source. "Sustainable energy is energy that in its production or consumption has minimal negative impacts on human health and the healthy functioning of vital ecological systems, including global environment" [15]. In short, "sustainable" means that "the resource renews itself at such a rate that it will be available for use by future generations" [16]. According to the International Energy Agency (IEA), renewable energy (19%) is currently the second largest energy source of electric power after coal (39%) [17]. Renewable energy sources include wind, solar, geothermal, hydropower, and biomass [17]. Considering only the United States, EIA reports that renewable energy provides approximately 8.6% (~3,500 trillion Btu) of the country's electric power [18].

In the transportation sector, ethanol derived from feedstocks such as corn & sugarcane is the most widely used fuel or fuel additive. Fuel ethanol in U.S. was produced at a daily average of 640 thousand barrels in September 2008 [19]. In terms of worldwide fuel ethanol production, about 60% is derived from sugar crops (e.g., sugarcane and corn), 33% from other crops, and 7% from synthetic sources [20]. In December 2007, the U.S. House of Representative and Senate signed the Energy Independence and Security Act, which mandates the production of 36 billion gallons of renewable fuel by 2022 [21]. Therefore, fuel ethanol production is expected to increase in the next few years.

2.3 History of Ethanol

Ethanol or ethyl alcohol has been used by humans since prehistoric times. It is one of the essential component in most alcoholic beverages, and 9,000-year-old pottery found in northern China provides evidence of the use of alcoholic beverages as far back as the Neolithic age [22]. The first isolation of ethanol as a relatively pure compound was obtained by Islamic alchemists, who also developed the art of distillation [23]. In 1796, the German chemist Johann Tobias Lowitz filtered distilled ethanol using activated charcoal, producing the first absolute ethanol. Twelve years later, Antoine Lavoisier describe the composition of ethanol as a compound of carbon, hydrogen, and oxygen; then Nicolas-Theodore de Saussure illustrated ethanol’s chemical formula [24]. Yet, it was only in 1858 that Archibald Scott Couper published its chemical formula [25]. Synthetically prepared ethanol was first made by Henry Hennel and S. G. Serullas in

1826. After two years, Michael Faraday successfully synthesized ethanol using acid-catalysis hydration of ethane, a technology still used today [26,27].

Ethanol was first used as fuel in 1826 when American scientist Samuel Morey developed an engine that utilizes ethanol and turpentine [28]. A German inventor, Nicholas Otto, followed him in 1860 with his famous development of the modern internal combustion engine [29]. The engine, later known as the Otto cycle, burns liquid fuels with air and uses the expanding hot gases to perform work [29]. Meanwhile, the United States had already used ethanol as fuel for lamps prior to the Civil War (1862). When Congress imposed liquor tax, ethanol became too expensive as fuel, thus people started using kerosene instead [27].

Five decades later, in 1908, Henry Ford released his first Model-T automobile. During the early 1900s, ethanol was massively produced as fuel (60 million gallons per year) due to the demands of World War I. Ethanol was added to gasoline to increase octane and reduce engine knocking. At the end of World War II, the use of ethanol for fuel has reduced due to low gasoline prices [4]. Ethanol only gained new interest during the release of Energy Tax Act of 1978 [30,31]. This act mandates the use of gasohol or gasoline-ethanol blends as transportation fuel [30]. The Tax Act was the action made by the government response to the oil embargo in 1973, the Iranian war in 1979 and the increasing environmental pollution caused by fossil fuel combustion [27]. The main goal of this law is to promote energy conservation, fuel efficiency, and renewable energy through tax credits [27]. Furthermore, the Energy Security Act of 1980 offered insured loans for small businessmen to support more ethanol production [30]. In the same year, Congress also imposed tariffs on imported ethanol to discourage buying of ethanol from foreign countries like Brazil. The U.S. government continued to support the use of ethanol as fuel by enacting a series of tax credits to encourage ethanol production.

In 1992, the energy policy act (EPACT) redefined gasohol as a blend of gasoline with at least 85% ethanol [30]. It also required car fleets to purchase alternative fuel cars that are capable of running both on E-85 (a blend of 85% ethanol and 15% gasoline) and gasoline [32,30]. In addition to EPACT, the Clean Air Act also strengthened the demand for ethanol production. The Clean Air Act of 1967, and later Clean Air Act Amendments of 1990, mandated the "wintertime use of oxygenated fuels in 39 major carbon monoxide non-attainment areas (areas where EPA emission standards for carbon monoxide had not been met) and required year-round use of oxygenates in 9 severe ozone non-attainment areas" [33]. Together with the implementation of the energy act, the tax incentives also increased from 40 cents/gallon in 1978 to 52 cents/gallon in 2003 [30].

Today, the statement of Henry Ford that ethanol is the fuel of the future is increasingly being realized. Currently, the United States officially has 180 ethanol biorefineries in operation at a capacity of 11.1 billion gallons/year; and 23 more plants are under construction [34]. With the flourishing research on lignocellulosic ethanol, the future of the industry appears promising.

As of November 21, 2008, it is noted that the world petroleum price has dropped to $47.05 per barrel as compared to $90.54 per barrel in November 2007 [2].

2.3.1 Why Fuel Ethanol?

Ethanol has been known to be relatively a cleaner and renewable source of energy. Wyman called bioethanol "the one fuel that has the potential to match the convenient features of petroleum at a lower price" [6]. Fuel ethanol is biologically produced from fermentation of glucose that is extracted from starch based crops such as corn, sorghum, cassava and sugar-rich plants such as sugarcane. Though it is not yet produced on large scale, ethanol can also be produced from lignocellulosic biomass such as switchgrass, corn stover, and agricultural residues [6,35,36]. Ethanol is considered a renewable fuel since it does not require exhaustible resources. Since ethanol is produced from plants that require CO_2 for respiration, the CO_2 emitted from vehicle combustion is counterbalanced, making the net CO_2 release zero [6]. Thus the carbon cycle balance is maintained, as shown in Figure 2.1. This will reduce the global warming effect on the planet. Although others might argue that the fossil fuel inputs needed to grow the plants and produce fuel ethanol should also be considered [37]. Yet, numerous studies have also shown that ethanol produced from corn and other feedstocks results in positive net energy balance (NEB – ratio of ethanol energy content to the fossil fuel used in the production) [7,38,39]. Furthermore, a recent study indicated 19-86% reduction in greenhouse gas emissions of fuel ethanol relative to gasoline [39].

A second reason for using ethanol as a fuel is to reduce dependence on foreign crude oil and avoid the difficulties encountered with fluctuating oil prices. In 1973, the so called "oil embargo" affected several countries when petroleum distribution to the west was discontinued. It has caused the crude oil prices to soar and high inflation to occur. Some crude oil suppliers disrupted the supply of oil hoping to control the U.S. foreign policy in the Middle East [16]. In response to the energy crisis, the United States started to switch to alternative sources such as solar, wind, biomass, and coal for power. The government also conducted a massive campaign on energy conservation and implemented daylight saving time (DST) to reduce energy consumption [27]. Because the United States had already started to develop alternative fuels, the Middle East eventually dropped the boycott. By 1980, petroleum prices decreased significantly and the U.S. started to import oil again [16]. Arguably, one of the lessons to be learned from this crisis is that "effective national security incorporates an energy policy that reduces heavy reliance on foreign cartels for energy resources" [16].

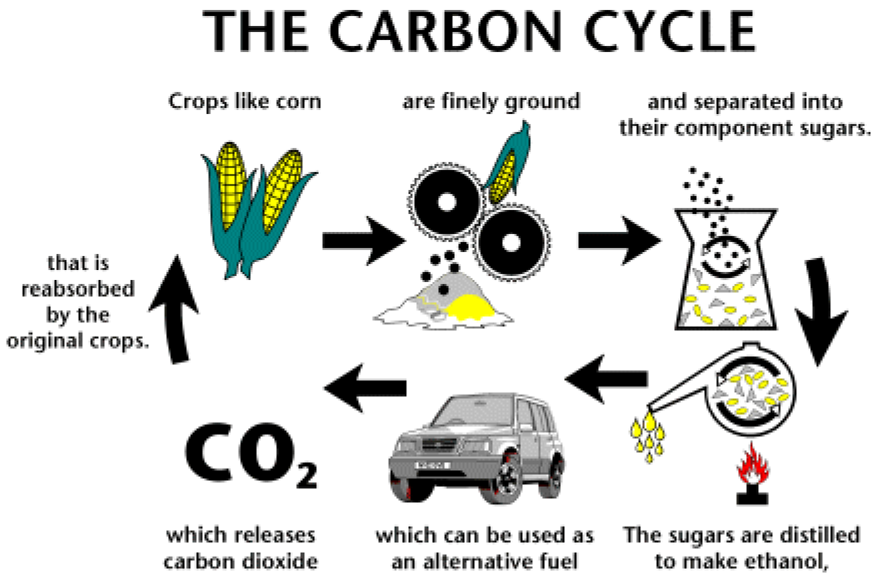


Figure 2.1 The Carbon Cycle
(Source: EIA. 2007. Kids Energy Page, "Ethanol–A Renewable Fuel.") [40]

The third reason for using ethanol for transportation fuel is its unique and versatile characteristics as fuel and as an additive. Ethanol is a relatively nontoxic and biodegradable compound with a higher octane number than gasoline [4,6]. It is known to supply oxygen to the fuel, decrease carbon monoxide generation, and keeps vehicle tailpipes free from unburned hydrocarbon [6]. Furthermore, ethanol has a "high heat of vaporization, low flame temperature, greater gas volume change, high specific energy, and high octane" [6]. These characteristics make ethanol ~15% more efficient than gasoline in a properly tuned engine [6]. These advantages can significantly offset the ethanol's lower energy content and lower mileage rating compared with gasoline [6].

2.3.2 Fuel Ethanol Production

Ethanol, commonly known as ethyl alcohol, grain alcohol, wine spirit, and cologne spirit, is a volatile, colorless, and flammable liquid compound. It is a straight chain alcohol with a molecular weight of 46.07 g/mol and a molecular formula of C_2H_5OH (Figure 2.2). Figure 2.2 illustrates the chemical structure of ethanol with green, red, and gray as carbon, oxygen, and hydrogen atoms, respectively. Ethanol has an agreeable but strong pungent smell and a bitter burning taste. It burns to form CO_2 and H_2O with a non-luminous blue flame and without soot formation. Relative physical properties of ethanol are tabulated in **Table 2.2**.

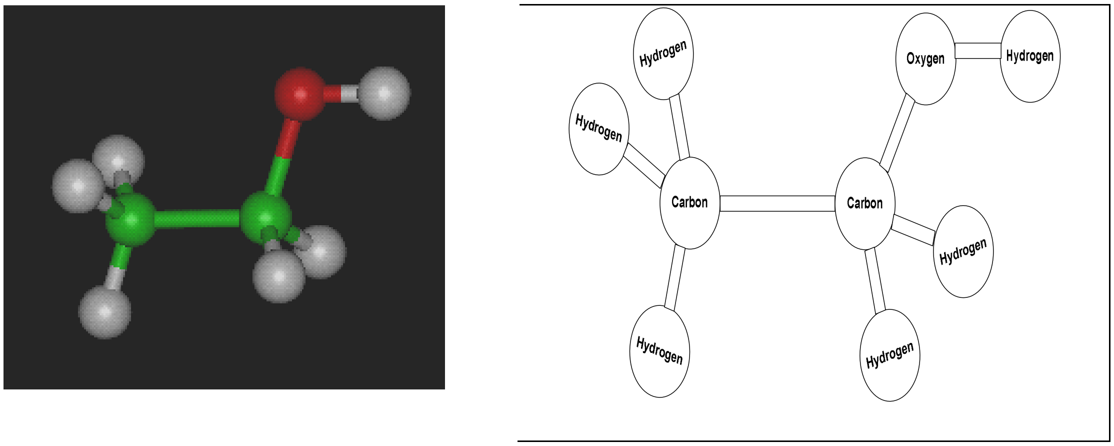


Figure 2.2 Molecular Structure of Ethanol
(Source: www.worldofmolecules.com/fuels/ethanol.gif) [41]

Table 2.2 Physical Properties of Ethanol [42]
(Source: Monick, J. A. 1968. *Alcohols, Their Chemistry, Properties and Manufacture,* Reinhold Book Co., A subsidiary of Chapman-Reinhold, Inc., New York. p. 106.)

	Absolute	**95% (by vol)**
Boiling point, °C	78.3	78.2
Flash point, °C	21	22
Freezing point, °C	-114.1	-
Heat of combustion of liquid, kcal/mole	328	-
Heat of fusion, cal/g	25	-
Heat of vaporization at boiling pt and 1 atm, cal/g	204.3	-
Ignition temperature (apparent) in air, °C	371-427	-
Density (20 °C) relative to water at 20 °C	0.7905	0.8038
Specific heat at 20°C, cal/g	0.579	0.618[a]
Surface tension at 20°C, dynes/cm	22.3	22.8
Vapor pressure at 20°C, mmHg	44	43
Viscosity at 20°C, cP	1.22	1.41
Refractive index η^{20}_{D}	1.3614	1.3651

[a] – at 23°C

Currently, ethanol in the United States is widely produced using corn as the main substrate. Typically, ethanol can be made via synthesis of ethylene or by a fermentation process, but for most fuel ethanol plants, the later is commonly used. Two methods are currently used to produce fuel ethanol from corn: dry milling and wet milling.

There are many types of corn *(Zea mays)* grown worldwide, but the commonly used for fuel ethanol are the yellow dent corn *(Zea mays* var. *indentata)*, also known as commodity corn. Dent corn has a broad, flattened seed with an average weight of 350mg. The corn kernel, as depicted in Figure 2.3, is made up of four main parts: hull or pericarp, endosperm, germ and tip cap. The endosperm and germ constitutes approximately 82.9% and 11.1%, respectively of the corn kernel's dry weight [43]. Table 2.3 lists the composition of the corn kernel [43]. The endosperm is mostly composed of starch, the corn's energy storage, and protein for germination [44]. The germ, which is considered as the only living part of corn, hold the essential genetic sequence, enzymes, vitamins, and minerals necessary for the corn's growth [44]. The pericarp or hull is made of a strong cellulosic material that resists water and water vapor. It also protects the kernel from microorganism and insects. The tip cap is the point where the kernel and cob are connected. It is the passage of water and minerals to the kernel [44].

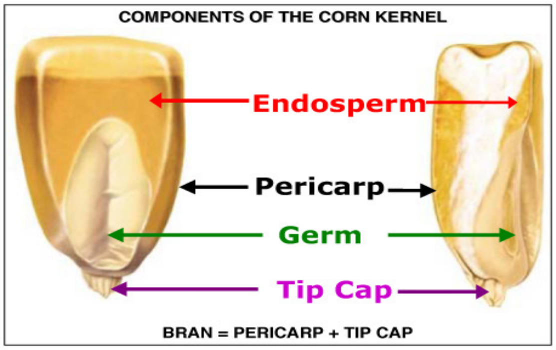


Figure 2.3 Components of the Corn Kernel
(Source: www.cerealprocess.com/images/corn-components.jpg) [45]

Table 2.3 Composition of Corn Kernel [43]
(Source: White, P.; Johnson, L. 2003. *Corn: Chemistry and Technology* American Association of Cereal Chemists., St. Paul, MN.)

Part		Mean composition of kernel parts (% dwb)					
	Mean percent of kernel(dry weight)	Starch	Fat	Protein	Ash	Sugar	Unaccounted
Endosperm	82.9	87.6	0.8	8.0	0.3	0.62	2.7
Germ	11.1	8.3	33.2	18.4	10.5	10.8	18.8
Pericarp	5.3	7.3	1.0	3.7	0.8	0.34	86.9
Tip Cap	0.8	5.3	3.8	9.1	1.6	1.6	78.6

Studies have shown that the typical corn in dry grind ethanol plants is composed of about 71.4% starch, 9.1% protein, and 4.04% oil [46]. Starch is a polymer made up of α-D-glucose units. It may contain traces of other constituents but they are negligible in comparison with the glucose monomers [47].

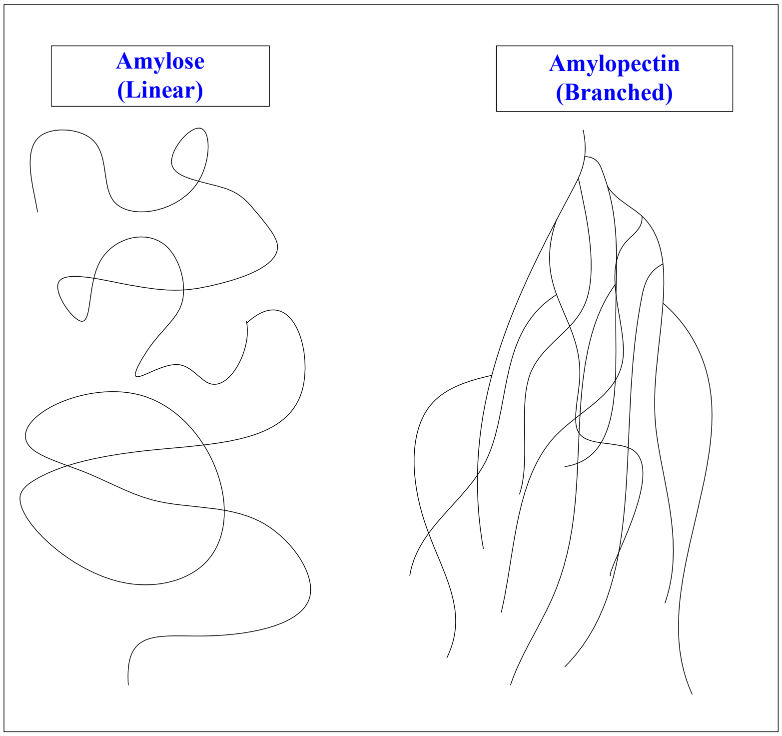


Figure 2.4 Amylose's Linear and Amylopectin's Branched Structure

Starch has two structural polymers, amylose and amylopectin. Amylose is assumed to be a linear polymer connected by α-1,4 glycosidic bonds while amylopectin is considered highly branched connected by both α-1,6 and α-1,4 glycosidic bonds (see Figure 2.4 & Figure 2.5). Amylose has an average molecular weight of approximately 250,000 g/mol (1,500 anhydroglucose units) [47]. Amylopectin is one of the largest molecules found in nature. The average molecular weight reaches as high as 1 billion g/mol (~500,000 anhydroglucose units) [47]. It is thought to be randomly branched, with 4-5% α-1,6 linkages [47].

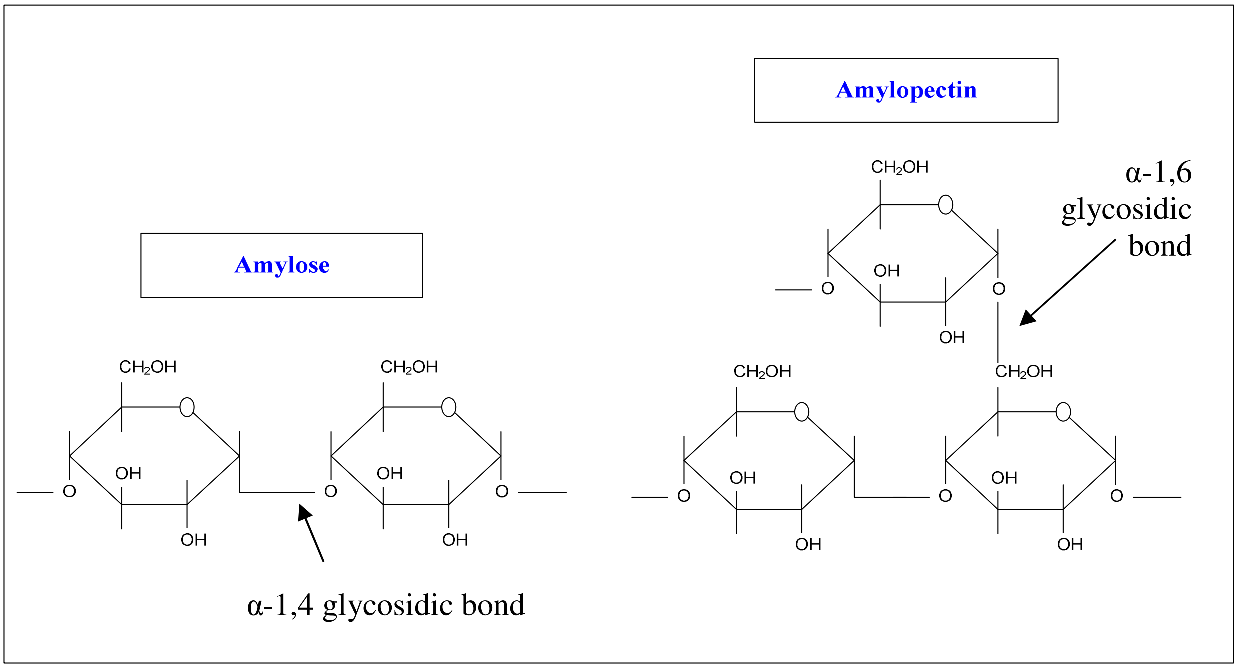
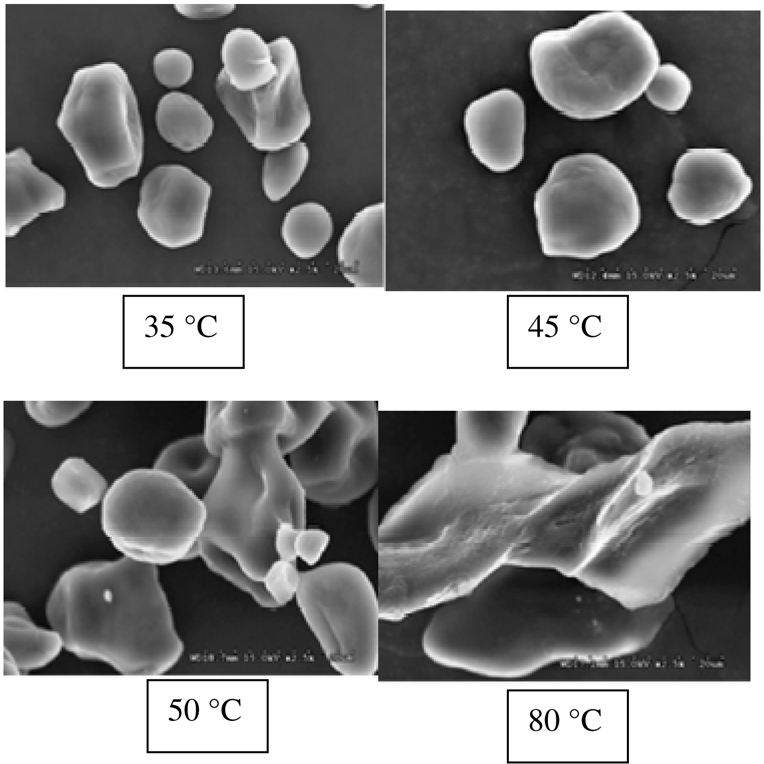


Figure 2.5 Glycosidic Bond Linkages of Amylose and Amylopectin Polymers

Gelatinization is an important mechanism in starch processing. Starch gelatinization is the loss of the semi-crystalline structure or the melting of starch crystallites in the presence of water. In more detail, it is the "complete destruction of the starch crystalline integrity as a function of moisture and temperature" [48]. Gelatinization can be achieved by either chemical or heat treatment. Nonetheless, heat treatment is more commonly used and is observed in this study. When starch is dissolved, the water freely penetrates into the granule. The starch granule can hold about 30% of its dry weight as water [47]. The granule will slightly swell, and approximately 5% volume change will occur. However, at this stage, the process is still reversible and the granule remains intact if dried [47]. As the temperature is increased beyond the starch gelatinization temperature, normally approximately 40-50°C, the granule begins to swell and releases its soluble amylose fraction, creating a three-dimensional matrix in the form of a gel [49]. The higher temperature heating of starch results in irreversible changes in the granule. It is known that "the soluble starch and the continued uptake of water by the remnants of the starch granules are responsible for the increase in viscosity" [47]. Ratnayake and Jackson [50] studied the stages of regular corn starch gelatinization at various temperatures (Figure 2.6).

Enzymes play an important role in the breakdown of starch. α-amylase or α-1,4-glucan-4-glucanohydrolase (E.C.3.2.1.1) [51] is known to attack both insoluble starch and starch granules held in aqueous solution. It endozymatically cleaves the 1,4-α-D-glucosidic linkages of polysaccharides containing three or more linear D-glucose chains [51]. The hydrolysis is random, yielding shorter maltodextrins as product. Therefore, the enzyme hydrolysis reduces the viscosity of the starch solution. β-amylase or glycogenase (E.C.3.2.1.2) is an exoenzyme that cleaves at the nonreducing end of starch and breaks every second α-1,4 glycosidic bond, producing maltose [47]. α-amylase is known to be more thermally stable than β-amylase [47]. Because both α-amylase and β-amylase could not hydrolyze α-1,6 glycosidic linkages, debranching enzyme such as glucoamylase, isoamylase or pullulanase is necessary to complete the starch hydrolysis. Glucoamylase or 1,4-glucoanohydrolase (E.C.3.2.1.3) is an exocleaving enzyme that sequentially removes one glucose unit at a time, starting from the nonreducing end, until the oligosaccharide hydrolysis is complete [52]. The enzyme is usually isolated from

Aspergillus niger and has an optimum pH of 4.2-4.5. It is the typical enzyme used in the saccharification process. Glucoamylase is very effective in cleaving single glucose units from starch polymer, but it is slow in hydrolyzing the α-1,6 branch point. As a result, an accumulation of isomaltose and pullulan can be found. To respond to this setback, debranching enzymes such as pullulanase, also known as amylopectin 6-glucanohydrolase (E.C. 3.2.1.41), or isoamylase (E.C.3.2.1.68) are used to supplement in the saccharification process. Both enzymes hydrolyze the α-1,6 linkages but not α-a,4 linkages.



35 °C

45 °C

50 °C

80 °C

Figure 2.6 SEM Images of Regular Corn Starch Gelatinization at Different Temperatures (Source: Ratnayake, W.; Jackson, D. "A New Insight into the Gelatinization Process of Native Starches," *Carbohydr.Polym.* 67:511-529) [50]

2.3.3 Dry and Wet Milling Process

Figure 2.7 illustrates a typical dry-grind process. Whole corn kernels are delivered to the plant, where it is ground by hammer mills to a specified particle size distribution. Particle size distribution has an impact in the following hydrolysis step. Large particle size distribution could save energy due to lesser milling power use but can result in poorer yield [53]. To compensate for the larger sizes, a more intensive liquefaction and saccharification can be done, which may require use of more steam energy and enzyme [53]. For a small particle size distribution, a higher yield can be achieved, but it will consume a larger amount of energy in milling and may increase the difficulty of downstream co-product recovery [53,54].

Ground corn is mixed with heated water to form the slurry. This step is often called mashing. Usually, ethanol plants also recycle a portion of thin stillage (backset) after centrifugation or a fraction of condensate after evaporation. This practice is used to recover a significant amount of excess heat from the distillation process. Moreover, the recycling step may promote gelatinization; enhance hydration, and decreased viscosity of the slurry. After mashing, one-third of the required alpha amylase enzyme is added to induce flowability of the mash. As the glycosidic bonds are broken, the viscosity of the slurry also decreases. Advances in biotechnology have resulted in improved α- amylase efficiency that is stable at temperatures as high as 105°C. These thermostable enzymes are often produced by a bacterial strain such as *Bacillus subtilis* or *Bacillus licheniformis* [53]. Dextrose equivalent (DE) of 4-5 units could be achieved [55]. Dextrose equivalent (DE) is a “measure of the percentage of glucosidic bonds that are hydrolyzed” [47]. Thus, DE of 100 indicates complete starch hydrolysis to glucose.

Corn slurry with temperatures 82-85°C is then pumped to hydro cookers, where it is exposed to steam at temperatures ranging from 121 to 148°C and a pressure of 100 psi. The main goal of jet cooking is to gelatinize the starch fraction of the corn, making it easier for enzymes to hydrolyze in the subsequent processes. Also, because of the very high temperature, jet cooking serves as a sterilization step to avoid bacterial contamination during fermentation. Though jet cooking is an energy intensive process (saturated steam at 121°C is about 1164 Btu/lb [56]), it only takes a few minutes. The slurry is immediately directed to the liquefaction tank, where the balance of the alpha amylase necessary is added. Ammonia and sulfuric acid are added to the tank to adjust the pH of the mash to the range of 5.9-6.2. The slurry will stay in the liquefaction tank for approximately 90 minutes before being pumped to the fermenters. A DE of 8-11 is usually obtained at the end of liquefaction [55].

Typically, ethanol plants use two types of enzymes to hydrolyze starch to glucose. α-amylase is the primary enzyme used in the liquefaction stage, while glucoamylase is used in saccharification. Although liquefaction produces soluble short chain oligosaccharides, another enzymatic hydrolysis is needed to further break down the oligomer into simple sugar. This step is known as saccharification.

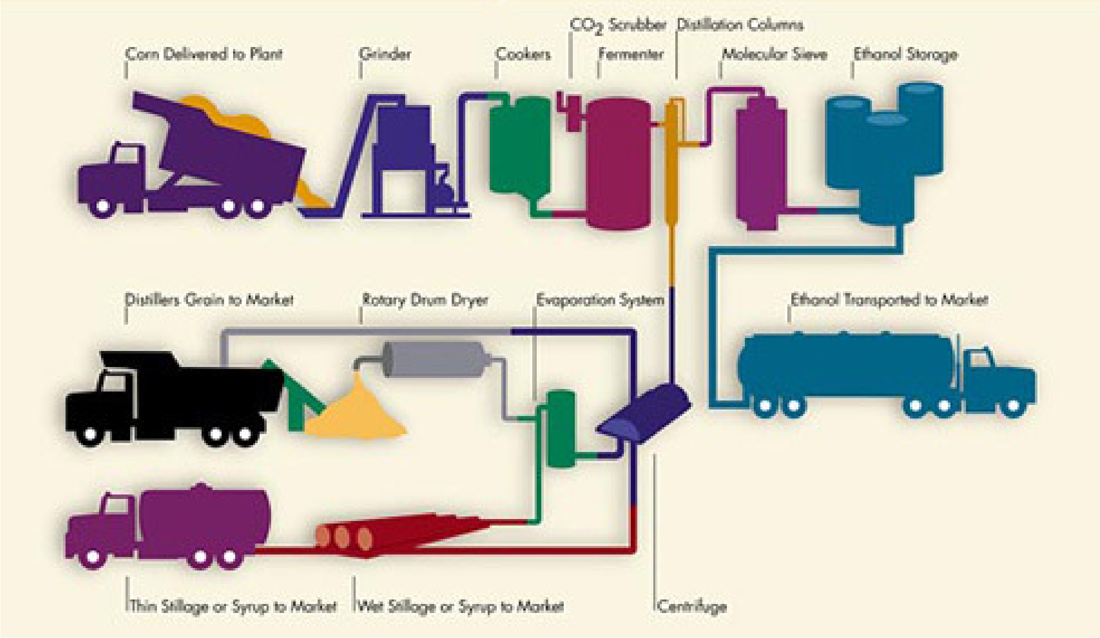


Figure 2.7 Dry Grind Milling Process (Source: http://www.ethanolrfa.org/) [34]

Saccharification is defined as the process of removing a single glucose unit from a soluble oligosaccharide [52]. This process is catalyzed by an exoenzyme, glucoamylase. For saccharification, the pH of the liquefied slurry is adjusted to 4.0-4.5 to favor the glucoamylase reaction. This pH adjustment is done to stop further action of α-amylase so that the slurry maintains an optimum oligosaccharide length for saccharification [52].

With the advancement of technology, modern ethanol plants have used the novel process known as simultaneous saccharification and fermentation (SSF), where glucoamylase is added with yeast [57]. Fermentation is conducted at lower temperatures (32-37°C), more favorable for the microorganism's growth. Although a slower saccharification rate occurs as temperature is lowered, the process is still advantageous because it eliminates glucose inhibition to yeast [57]. Additionally, because this process is conducted in one unit, it minimizes the capital cost for additional vessels and saves residence time from a dedicated saccharification process [53].

During fermentation, *Saccharomyces cerevisiae* or closely related yeast strains are used to anaerobically convert glucose to ethanol. The chemical equation below summarizes the reaction in which one molecule of glucose is converted to two molecules of ethanol and two molecules of carbon dioxide. Therefore, 100g of glucose will theoretically produce 51.1g of ethanol.

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2 \qquad \textbf{Eq. 2.1}$$

Using modern ethanol plant facilities, ethanol yields can approach 95-96% theoretical conversion, on a starch basis [53]. This assumption excludes the glucose fraction used to propagate yeast cells at the initial stage of fermentation. Yield can be reduced due to the formation of products such as glycerol, succinate, and higher molecular weight alcohols (isobutanol, propanol) [53]. Moreover, low-level contamination from *Acetobacter* and *Lactobacillus* bacteria can result in acetic acid and lactic acid production. Traditional fed-batch fermentation processes takes about 48-72 hours to complete. The CO_2 released during fermentation can be collected and sold to beverage companies for carbonating softdrinks and to dry ice producers, although this is generally uneconomical.

After fermentation, the fermented beer, including the unconverted solids, is fed to the distilling columns for ethanol recovery. Distillation is a process of separating the components of the liquid feed into several fractions using the differences in the components' boiling points. As the liquid evaporates, the gas is then condensed back to liquid in a purer form. The distillate is the desired product of distillation, and the bottoms contain all the nonvolatile components of the feed. The distillate is passed through molecular sieves to dehydrate the solution to 200 proof ethanol. The dehydrated ethanol is then blended with approximately 5% gasoline as denaturant to render it unconsumable and shipped to gasoline stations.

Meanwhile, the bottoms, also known as whole stillage, which contain residual starch, spent yeast, and other unfermentable materials, are centrifuged. This process separates the solids (course grains) from the liquid (centrate). Centrate, also known as thin stillage and backset, are either recycled back to the slurry tank or concentrated further using evaporation. The evaporated water is condensed and recycled back in the process while the dewatered solids (syrup) are added to the solids (coarse grains). The condensate and backset are typically recycled as "cooked water" to utilize the heat and aid mashing of the corn. Syrup and course grains are combined and dried to produce dried distillers grain with solubles (DDGS). This is primarily used as an animal feed for ruminants.

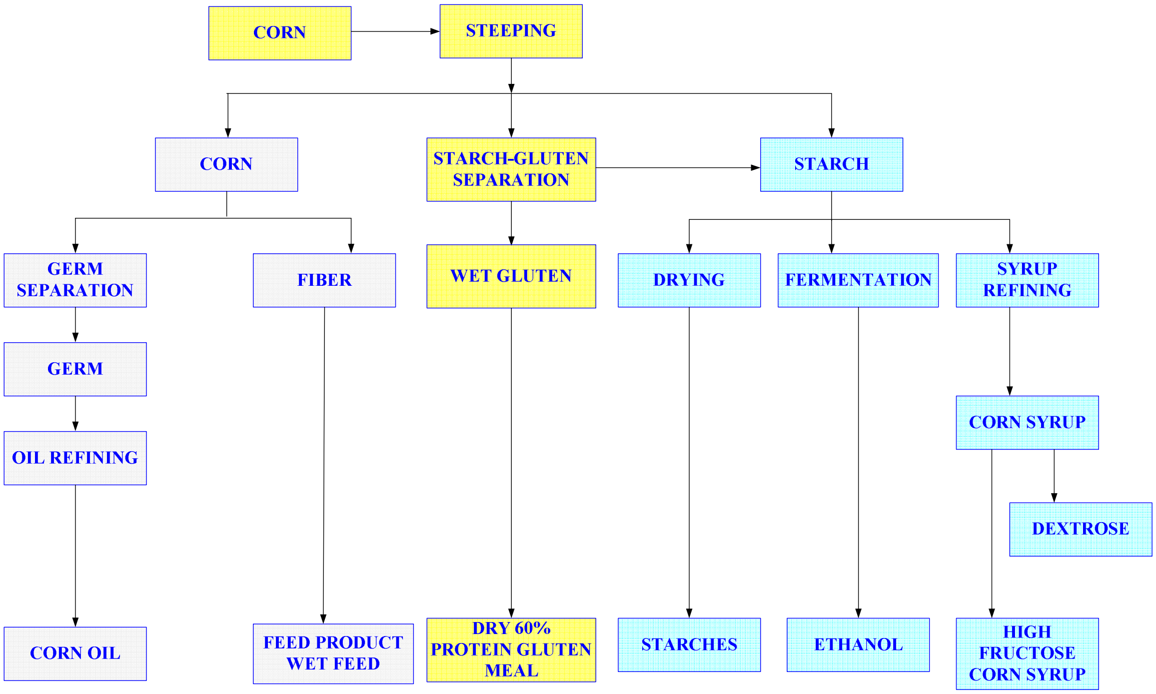


Figure 2.8 Wet Milling Process (redrawn from http://www.ethanolrfa.org) [34]

In contrast to dry-grind milling, the wet milling produces numerous products, namely corn oil, gluten meal, ethanol, high fructose corn syrup, and gluten feed. Figure 2.8 describes the schematic processes for wet milling plants. Wet milling process starts with the steeping of corn kernels in sulfur dioxide solution for 22-50 h [58]. The lactic acid ($C_3H_6O_3$) and sulfur dioxide (SO_2) in the steepwater soften the pericarp (outer covering) of the corn and release the starch and other components. After steeping, the corn is separated from the steepwater and conveyed to a series of milling processes to free the starch from the germ and hull. Corn oil and corn gluten meal is extracted from the germ and hulls using various extraction processes such as centrifugation, evaporation and hydroclonic separation [34]. The corn oil removed from the germ can be sold commercially or further processed to produce biodiesel. The gluten, which is mainly protein, is processed to produce corn gluten meal. It is usually used as poultry feed ingredient. Meanwhile, the starch fraction is washed with water in a series of hydrocyclones. It can be further processed in three different ways: fermented to ethanol, sold as dried starch, or processed into high fructose corn syrup. The fermentation process is similar to dry grind milling operations. Wet milling is advantageous over dry milling in terms of lower net corn cost, higher component recovery, and higher co-product value. However, the drawback of wet milling plants include higher capital cost, larger energy consumption and lower overall ethanol yield due to loss of starch in the pretreatment stages [53].

2.3.4 Pros and Cons of Ethanol as Fuel

Several studies suggest that ethanol is not a viable renewable energy source because of numerous issues such as the negative net energy value (NEV), the harmful environmental effects and the diversion of a food supply to fuel [59,60,61]. “Net energy value (NEV) is the energy content of ethanol minus the fossil energy used to produce ethanol” [7]. There are considerable amount of reports depicting the estimation of net energy balance. Table 2.4 presents the different net energy value and output to input ratio of ethanol as a fuel. Pimentel [59] indicated that 99,119 Btu is required to produce a gallon of ethanol, resulting in a net energy loss of 22,119 Btu per gallon. In terms of the output to input energy ratio, this is equivalent to 0.78, a value lower than that reported by others [7,62,63]. In contrast, Kim and Dale [63] showed a 79% net energy gain from every gallon of ethanol. From a historic point of view, it appears that the energy required to produce one gallon of ethanol has decreased over time. This could be due to improvements in processing, planting and harvesting technology as well as an increase in crop yields.

Table 2.4 Net Energy Value of Different Studies

Authors	Net Energy Value (Btu/gal ethanol)	Output/Input (based on LHV)
Pimentel (2001) [37]	-33,562	0.69
Pimental (2003) [59]; Pimentel and Patzek (2005) [60]	-22,119	0.78
Keeney and de Luca (1992) [64]	-8,438	0.90
Graboski (2002) [65]	18,329	1.32
Shapouri et al. (2002) [7]	21,105	1.34
Wang et al. (1999) [66]	22,500	1.42
Lorenz and Morris (1995) [67]	30,589	1.67
Kim and Dale (2002) [63]	33,585	1.79

"It has been long recognized that computations for net energy value are highly sensitive to the assumptions of both system boundaries and key parameter values" [68,69]. Differences among these studies are related to various assumptions such as corn yields, fertilizer application, ethanol conversion technologies, co-product credit evaluation, and other sources of energy inputs. Farrell et al. [69] evaluated six published studies [7,60,62,65,70,71] and developed the Energy and Resources Group Biofuel Analysis Metamodel (EBAMM) to directly compare the data of the two. Farrell, et al. [69] indicated that studies of Pimentel & Patzek [60] and Patzek [70] stood out among the six studies because they reported negative net energy values and relatively high greenhouse gas emissions from ethanol production. Farrell et al. [70] found that these two studies have incorrectly assumed that ethanol co-products (DDGS, corn gluten, corn meal, corn oil) should not be credited in any part of the energy input values. Also, the study [69] stressed that the input data used in the two studies were old and unrepresentative of current processes. These observations were also mentioned in the report of Shapouri et al. [7].

Several studies have also discussed the impact of ethanol on the environment. Pimentel [59] reported that U.S. corn production causes soil erosion, water pollution from herbicide and insecticide use, and groundwater table contamination due to nitrogen leaching from fertilizers. On the optimistic side, the study of Shapouri et al. [7] showed that with the progress in technology, fertilizer use in the grain production has declined. Additionally, the manufactures of agricultural chemicals have become more efficient, so that farmers have gained significant energy savings on nitrogen and phosphorus. It has substantially reduced the use of nitrogen, potassium, and phosphate. Furthermore, another study has also pointed out that implementing energy saving practices and methods in ethanol plants has reduced the overall energy consumption [72]. Overall, it signifies a promising outlook for ethanol in the future.

Because of various economic and energy value uncertainties, ethanol production in the United States remains controversial, both in the scientific and in the economic world. In a recent publication by the USDA [57], it was reported that new technologies are slowly emerging that could reduce energy requirements and ethanol production costs. A few of the technologies mentioned involved process improvements in separation technologies, fermentation, distillation, and control systems. Another important technology is the development of new enzymes that require less heat for liquefaction and saccharification, which is one of the most promising energy saving developments. Although these enzymes are not currently used commercially, they offer a great potential for reducing the amount of energy used to produce ethanol. Because of contradicting publications and ambiguities in the energy balance measurements, any improvement in the efficiency of the production of ethanol would help secure its viability as a renewable energy source.

2.4 Ultrasonics: A Potential Pretreatment Process

Traditionally, dry milling plants used natural gas and coal as the process fuels for production. These fuels are used to raise steam for jet cooking, distillation, drying, and evaporation. The jet-cooking step is an important part of the process as it aims to induce starch gelatinization, aid enzymes in the breakdown of starch, and sterilize the corn slurry in preparation for fermentation. The jet cooker, also known as the hydro-cooker, uses steam at pressures of 1 MPa (150 psi) and temperatures ranging from 121 -148°C (250 to 300°F). The steam in this process has direct contact with the corn slurry to attain a better cooking effect. It is noted that 1 pound of saturated steam at 121°C has about 1,164 Btu (1,228 kJ) of thermal energy [56]. In a state-of-the-art dry milling plant with 50 million gallon per year production, one gallon of ethanol requires 17,200 Btu (18,146 kJ) using natural gas and 31,400 Btu (33,128 kJ) using coal of fuel energy for steam production [73]. Assuming 5% of the process steam is used for jet cooking (approximately 860 Btu (natural gas) and 1570 Btu (coal) per gallon ethanol), this represents a significant amount of energy. Therefore, enhancements in process efficiency will result in considerable improvements in the annual energy consumption. Additionally, it will enhance the net energy value (NEV) of ethanol production from corn. In aiming to reduce the energy usage, this study proposes ultrasonic treatment as a potential pretreatment method that requires significantly less energy input than jet cooking.

2.4.1 Ultrasonics

Ultrasound is defined as sound waves at frequencies beyond the human hearing range (20 kHz) [74]. The range of human hearing for a young person can vary from 20 Hz to 20 kHz [75]. “Sound is transmitted though a medium by inducing vibrational motion of the molecules through which it is traveling” [74]. There are two distinct classifications of ultrasound, which can be broadly divided according to a large frequency range. The first type includes low amplitude sound, commonly known as “low power” ultrasound. Amplitude is the peak oscillation displacement of the sound wave. Low power ultrasound, within the typical application range of 2-10 MHz, is used “for analytical purposes to measure the velocity and absorption coefficient of waves in a medium” [75]. It is often used in medical imaging, chemical analysis, medical diagnostics, nondestructive testing and therapeutic medicine. The other classification of ultrasound is called “high power” ultrasound. It usually involves lower frequencies (20-100 kHz) and high amplitudes (~12-320μm-range based on this study). At this range, greater acoustic energy can be generated, inducing cavitation in liquids [75]. It is used for cleaning, cutting, metal and plastic welding, cell disruption, emulsification, and crystallization.

“When ultrasound wave propagates in a medium such as a liquid or slurry, it produces cavitation. Cavitation generates powerful hydro-mechanical shear forces in the bulk liquid, which disintegrate nearby particles by extreme shear forces” [5]. Another important ultrasonic effect in liquids is acoustic streaming. Rooney [76] defined acoustic streaming as a “time-independent flow of fluid induced by a sound field”. Although this study may find that the “time-independent flow” may only apply to the temperature controlled system. Studies have shown that acoustic streaming has enhanced heat and mass transfer, reaction rates, emulsification, and depolymerization [76].

2.4.2 Beneficial Effects of Ultrasonics

Ultrasonic technologies have been extensively used in diverse industrial applications for decades, especially in carbohydrate chemistry. Kardos & Luche [77] reported that the first ultrasonic application on starch was done by Szent-Gyorgi [78] and Szalay [79] in 1933. After 7 years, Ono [80] also reported that potato starch particles were successfully disintegrated by ultrasound. The effect of ultrasound in starch varied depending on the starch concentration, characteristics of the dissolved gas present, viscosity, reaction temperature, and starch molecular size [80]. A number of studies reported that as starch concentration increases, the ultrasonic efficiency decreases [8,10, 57,81]. One possible reason could be the reduction of the relative power density (W/g) delivered at higher starch concentrations. Some studies also indicated that dissolved gases influence the effectiveness of ultrasonics in starch disintegration [80]. Dissolved oxygen was established to be more favorable than dissolved hydrogen [80,82]. Meanwhile, carbon dioxide showed very weak results, and under vacuum virtually no ultrasonics effect was found [82]. Further on, studies with temperature control systems indicated an increasing rate of depolymerization at or above the starch gelatinization temperature [81]. Seguchi, et al. [10] also found starch molecular weight reduction due to ultrasonics.

Isono, et al. [81] defined ultrasonic depolymerization as a “nonrandom process where chain scissions near the center of the largest molecules are favored”. In other words, ultrasound produces fragments of molecules and was observed to cut near the midpoint of the polymer chain [83]. Ultrasound is capable of degrading macropolymers such as starches and allows the degradation of the larger molecular weight distribution in the polymers. Studies have confirmed this mechanism using starch as substrate [80,81]. The rate of degradation depends mainly on the extent of the ultrasound treatment, the physical and chemical properties of the polymer, the concentration of the solution and the intensity or amplitude of the ultrasound [80,81,83]. In these cases, cavitation breaks the bonds between the chain molecules and breaks them into smaller molecules. However, Basedow and Ebert [83] disagreed and reported that in terms of the reversible viscosity reduction due to ultrasonics, the mechanism could be explained that “ultrasound caused the dispersion of aggregates, rather than the breakage of chemical bonds”.

In starch depolymerization, the first step is to gelatinize the granule to release the starch from its semi-crystalline form. Gelatinization is a mechanism of starch that occurs when starch loses its native semi-crystalline structure in the presence of water. This process can also be achieved by ultrasonics. This study found that the synergistic reaction of ultrasonic cavitation and thermal effects due to ultrasound are responsible for the starch gelatinization. However, it is noted that in other depolymerization studies (nitrocellulose, polystyrene and cellulose nitrate), it was found that “direct thermal effects of ultrasound are not responsible for the degradation of polymers” [83,84,85]. Therefore, the nature of the polymer could be one of the factors that dictate the depolymerization mechanism during ultrasonic treatment.

If this study is to apply ultrasonics during saccharification, the effect of ultrasonics on enzymes is important. There have been a number of interesting studies on the stability of enzymes under sonication. At low acoustic power, some enzymes are not deactivated, whether immobilized or free in solution [77,86]. However, at high ultrasonic intensity, enzymes can also be denatured [11,87]. In a study by Wood et al. [88], intermittent ultrasonication was found to increase ethanol yield and enhance enzymatic reaction in the simultaneous saccharification and fermentation (SSF) of mixed waste office paper. The study also showed that in the ultrasonic assisted experiments only half the amount of enzyme used in the unsonicated SSF was required to produce a similar ethanol yield. These finding suggests that ultrasonics can be an attractive and cost effective method for reduction in the enzyme used and potential energy savings.

Another possible benefit of ultrasonics is sterilization. Bacterial lysis with ultrasonics has been studied as an alternative method to conventional sterilization. Ultrasound has been used in cell disruption of microorganism in waste activated sludge and as intracellular extraction technique [75,89]. However, using ultrasound for complete sterilization requires very high energy intensities [90]. Therefore, studies have coupled ultrasound with other sterilization technique such as heat and chemical treatment to provide complete destruction of microorganism [90,91]. Due to the use of moderate heat and reduced chemicals, the coupled ultrasound sterilization method is a better option over conventional heat or chemical treatment alone. It also has shorter operating time and lower overall energy usage.

2.4.3 Sonochemistry

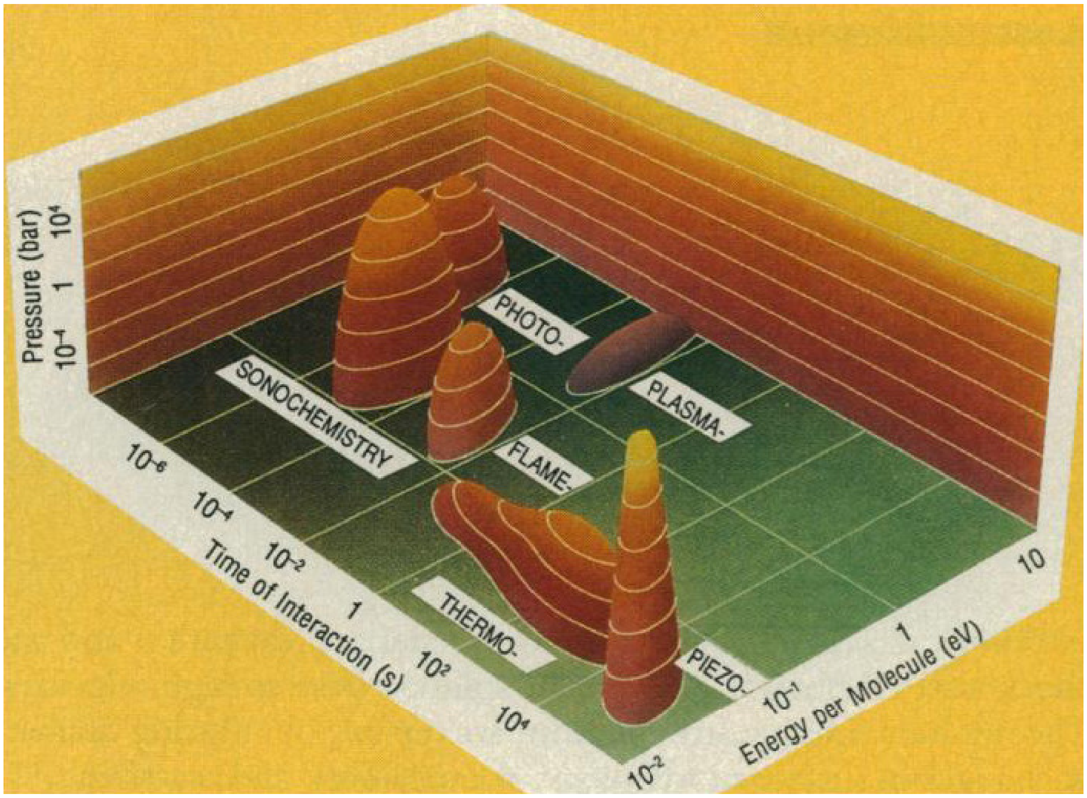


Figure 2.9 Chemistry: Interaction of Energy and Matter (Source: Suslick, K. 1990. "Sonochemistry," *Science* 247(4949): 1439-1445.) [92]

In order for many chemical reactions to occur, the addition of external energy is required [92], commonly known as the activation energy. Figure 2.9 is a representation of various ranges where energy and matter interact in chemistry. As seen, sonochemistry has a unique interaction of energy and matter which differs from traditional sources of energy such as heat and light in terms of time of interaction, pressure, and energy per molecule [92]. Sonochemical reactions occur in a shorter time range at higher pressures and energy dissipation. Sound waves do not chemically interact directly with the molecules but instead produce the energy indirectly from the complex phenomenon called cavitation

[92]. Acoustic cavitation is the main factor responsible for most sonochemical reactions. Within the treated liquid, “one can create temperature of the sun’s surface, the pressure of deep oceanic trenches, and the cooling rate of molten metal splatted onto a liquid-helium-cooled surface” [92]. The physical and chemical effects of ultrasound have involved significant enhancements both in stoichiometric and catalytic reactions [75].

2.4.4 Ultrasonic Equipment

Ultrasound is generated using a transducer or converter. Ultrasonic transducers are designed to convert electrical energy into mechanical energy at high frequency [74]. There are two main types of electromechanical transducer, piezoelectric and magnetostrictive [74]. Magnetostrictive transducers use the ability of selected materials, e.g., nickel, to change size when placed in a magnetic field. Magnetorestrictive transducers are often used in large industrial processes because it is durable, robust and supply large driving forces [74]. However, this technology has a low electrical efficiency (<60%) due to heat losses and limited frequency range (max~100 kHz), resulting to limited application [74]. Since the piezoelectric transducer is commonly used and is also the transducer used in this study, magnetostrictive transducers will not be discussed further in this dissertation.

Piezoelectricity, meaning “electricity through pressure”, was first discovered by Pierre Curie in 1880 [93]. Today, piezoelectric transducers often use ceramic material containing lead zirconate titanate, also known as PZT [74]. If a charge is applied across the piezoelectric material, it will either expand or contract depending upon the region of the applied charges [74]. This effect will create the mechanical vibrations and transmit it to the medium with which it is connected [74].

In simple terms, using small mechanical vibrations, ultrasonics can deliver large amount of mechanical energy [94]. However, the vibrational amplitude or displacement is limited by the transducers maximum allowable stress, frequency and other tooling designs [94]. Typically, for given amplitude, higher frequencies generate higher velocities and therefore greater power dissipation [95]. However, the available power from higher frequency transducers is usually lower compare to lower frequency transducers. Due to the material property of the piezoelectric disc, transducer size is often reduced as frequency increases, which can also limit the tooling design of the system [94]. Additionally, piezoelectric materials are temperature sensitive; therefore transducers must be cooled when used for long times and at high power dissipation [74]. Various manufacturers offer ultrasonic units with a wide range of frequency, depending on the application. For most starch sonochemistry, studies have used various ultrasonic frequencies from 20 to 470 kHz [5,9,10,77,80,81,82]. Although others have also tried using higher frequencies, yet these studies recommended the use of lower frequencies for better physical and chemical reactions [80,82].

Although cavitation is responsible for most chemical reactions in sonochemistry, it also has some drawbacks. One of the disadvantages of this system is the power limitation. As mentioned earlier, when the frequency decreases, the transducer size increases, which will lead to doubling the size of the system for lower frequencies [74]. Sometimes, due to high power dissipation, heat is generated in the transducer, and prolonged heat will eventually damage the transducer [74]. Thus, the maximum power capacity of a system may also be affected by the size of the transducer [96].

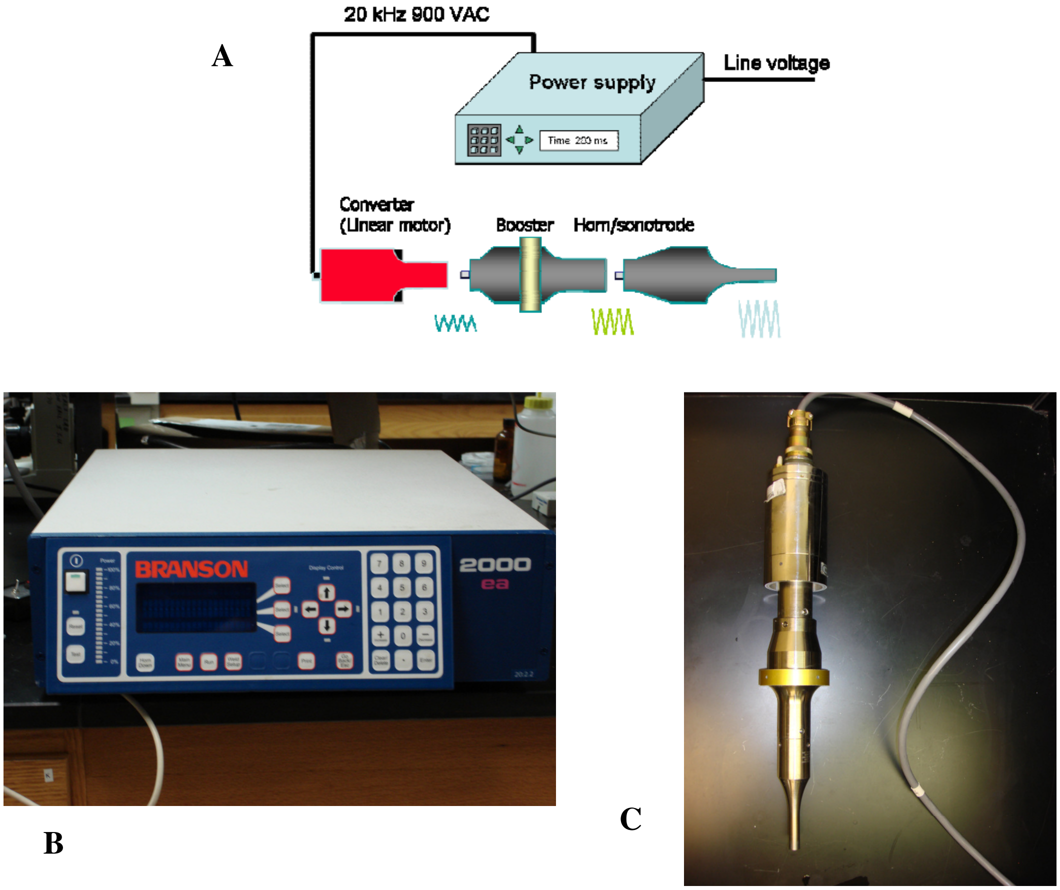


Figure 2.10 Ultrasonic Equipment Schematic Diagram (A) (Source: http://www.bransonultrasonics.com/) [97] and Images of Ultrasonic Equipment (B,C) Used in Experiments

Another disadvantage is cavitation erosion. Cavitation erosion in metals occurs as a result of the violent collapse of microbubbles near the surface of the metal [98]. As the bubble implodes, it produces shock waves and liquid jets with enough force to deform and fracture a metal [98]. Therefore, in most industrial applications the ultrasonic horns/tips are periodically changed when they are eroded. Although ultrasonic erosion also occurs in non-metallic materials, it has not been largely explored compared to metallic erosion [98].

A standard ultrasonic system (as well as the system used in this dissertation) is shown at Figure 2.10. The system is composed of three main parts: transducer, booster and horn (Figure 2.10A). The transducer or converter is connected to the power supply (Figure 2.10B), where the power supply converts the available electrical voltage line into high frequency electrical energy at the same frequency provided by the power supply [95]. The transducer changes the electrical energy into mechanical energy (Figure 2.10C). The amplitude can be adjusted from 0 to 20 μm by adjusting the voltage delivered by the power supply. The booster, which is coupled to the horn, can increase or decrease the amplitude of the mechanical vibrations, depending on the size and shaped of the booster/horn. The horn can also further amplify the amplitude depending on the gain of the horn.

2.4.5 Cavitation

Acoustic cavitation is a well-known mechanism of sonochemistry. Cavitation was first observed by Sir John Thornycroft and Sidney Barnaby [74,99]. "They were called to investigate the poor performance of a new screw driven destroyer, HMS Daring, the ship did not reach the high speeds expected. The problem was traced to an incorrect setting of the propeller blades resulting in inefficient thrust." [74] Mason [74] further explained that "as a result of this study, it was found that the rapid motion of the propeller blades through the water one face produced thrust, as expected, but the trailing edge produced sufficient ‘negative’ pressure in the water to pull molecules apart and create tiny microbubbles (cavities)”. The same “cavitation” principle was used by the Germans during World War II with their “passive sonar” [74]. The method uses cavitation noises due to propellers to detect submarines [74].

Cavitation is a phenomenon of bubble formation in liquid when negative pressure is applied [74,100]. Cavitation may be divided into two categories, transient (inertial) and stable (non-inertial). “Transient cavitation bubbles are voids, vapor filled bubbles, believed to be produced using sound intensities in excess of 10 W/cm^2.” [75] Meanwhile, stable cavitations are non-linear oscillations which are produced in relatively lower intensities ranging from 1-3 W/cm^2 [75].

As the mechanical vibrations of ultrasound propagate in liquid media, it compresses and stretches the liquid and “generate a repeating pattern of compression and rarefaction” (Figure 2.11) [74,101]. As the oscillation continues, it creates negative pressure within the liquid, breaks the liquid apart and eventually form voids within the media which is commonly called as microbubbles [101]. These bubbles are said to initiate during the rarefaction cycle [74]. “The process by which dissolved gas in the liquid is converted into free gas in the form of bubbles by the action of the sound field is called rectified diffusion.”[102] As the oscillation continues, the microbubbles will grow to unstable size and eventually collapse violently dissipating large amount of energy with localized temperature of 5000 K and pressures of 180 MPa [92,101].

Ultrasonic cavitation is said to occur in three steps: nucleation, growth and collapse (Figure 2.12) [83,92]. Acoustic cavitation is affected by various factors such as viscosity, surface tension, vapor pressure, dissolved gases, temperature and frequency

[74]. Cavitation is usually difficult in viscous liquids because it will have high resistance to shear [74]. Similarly, when the liquid has high surface tension, there will be lesser dissolved gases, thus cavitation will also be difficult [83].

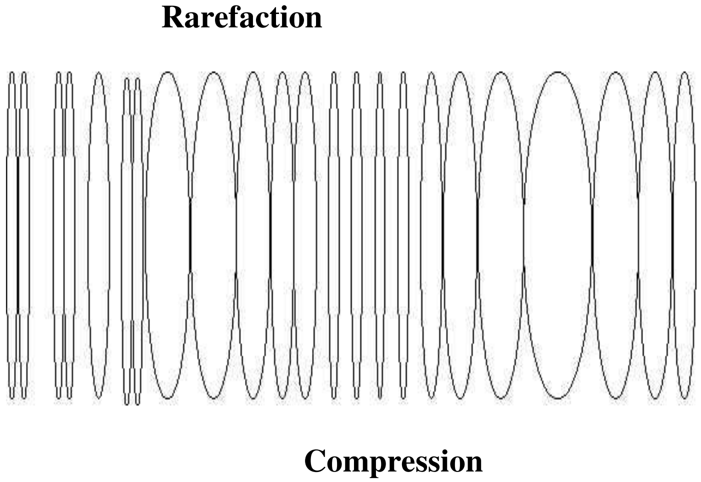


Figure 2.11 Compression and Rarefaction of Ultrasound Waves (Source: University of New Mexico. 2008. Available online at www.unm.edu/~abqteach/Physics%20CU/01-01-17.jpg) [103]

It has been found that as ultrasonic frequency increases, cavitation intensity decreases, as well as power dissipation. Mason and Lorimer [75] explained that “at very high frequency, where the rarefaction and compression cycles are very short, the finite time required for the rarefaction cycle is too small to permit a bubble to grow to a size sufficient to cause disruption of the liquid.” They further added that “even if a bubble was to be produced during rarefaction, the time required to collapse that bubble may be longer than is available in the compression half cycle” [75]. Therefore, the resulting cavitation effects will not be as effective at low frequencies [75].

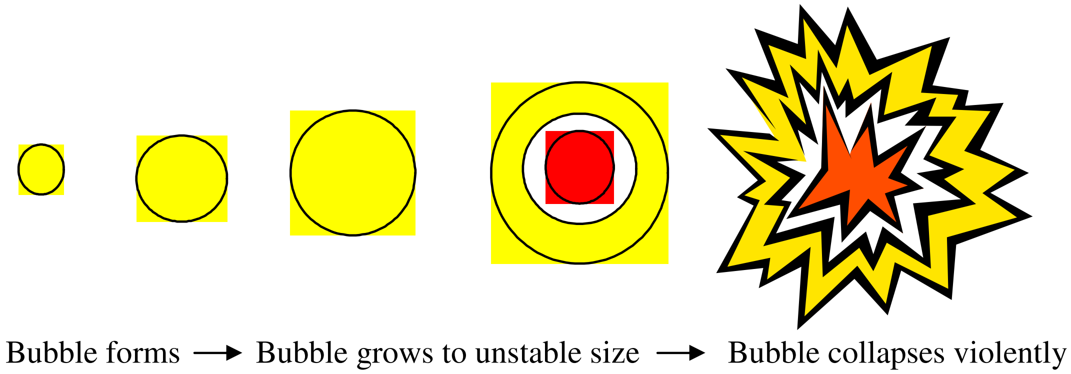


Figure 2.12 Development and Collapse of Cavitation Bubbles

2.4.6 Acoustic Streaming

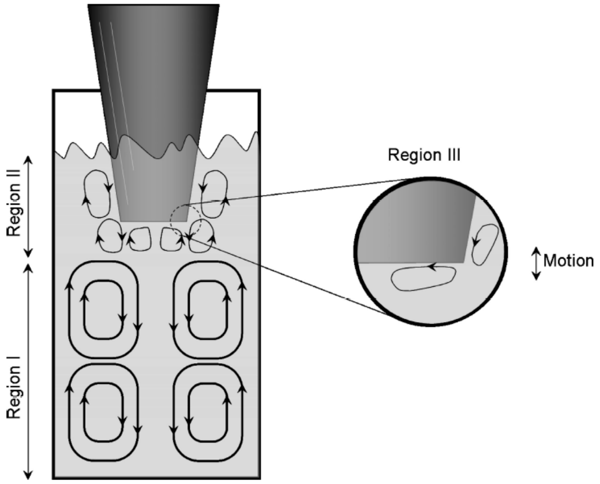


Figure 2.13 Illustration of Acoustic Streaming (Source: Khanal, S.K.; Grewell, D.; Sung, S.; van Leeuwen, J. 2007. "Ultrasound Applications in Wastewater Sludge Pretreatment: A Review" ***Crit. Rev. in Env. Sci, & Tech.*** **37:277-313 [101])**

Acoustic streaming was first studied by Lord Rayleigh in 1896 [104]. His experiments with standing waves showed "a regular array of eddy flows, each separated by a quarter wavelengths" [76]. He found that the change in the "streaming velocity was greatest at the walls and smallest at the middle region of the channel" [76]. After his discovery, many models have been proposed to explain this mechanism.

Studies have shown that "acoustic streaming occurs in four symmetrical counter-rotating circulations" [76,105]. Figure 2.13 have shown 3 types of streaming namely Eckart streaming, Rayleigh streaming, and Schlichting streaming which corresponds to region 1, 2 and 3, respectively [101]. Many have also reported that acoustic streaming velocity is dictated by ultrasonic intensities, fluid properties, ultrasonic frequencies, and amplitudes [106,107,108]. Clarke, et al. [106,109] cited that "acoustic streaming velocity decreases with increasing fluid viscosity and increases with increasing attenuation". The study [109] used acoustic streaming to investigate *in vivo* hematoma diagnosis.

Today, acoustic streaming is used effectively in improving rate transport processes such as mass and heat transfer, enhanced agitation, and changes in biological cells. A few important contributions due to acoustic streaming included the studies of Hughes and Nyborg [110] on the hemolysis of erythrocytes, Nightingale et al. [108] on breast lesion diagnosis and Dyer and Nyborg [111] on motions induced in plant cells.

2.5 Summary

Growing concerns about global warming, economic stability, sustainability, and national security, have led to resurgence in the development of alternative fuels, such as ethanol from corn. However, in order for these alternative fuels to become viable sources of energy, their production must be improved in terms of economics and net energy value. This study proposes the use of ultrasonics as a potential substitute or enhancement for jet cookers during the pretreatment of corn slurry. The research was conceptualized because jet cookers consume a significant amount of energy (~100 BBtu/yr) in the production of ethanol. If ultrasonics could perform as effectively as jet cookers, then it would significantly reduce the energy consumption of the ethanol plant.

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CHAPTER 3: ULTRASOUND ENHANCED GLUCOSE RELEASE FROM CORN IN ETHANOL PLANTS

A paper published in *Biotechnology and Bioengineering*

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Abstract

This work evaluated the use of high power ultrasonic energy to treat corn slurry in dry corn milling ethanol plants to enhance liquefaction and saccharification for ethanol production. Corn slurry samples obtained before and after jet cooking were subjected to ultrasonic pretreatment for 20 and 40 seconds at amplitudes of vibration ranging from 64 to 107 μm_{pp}(peak to peak amplitude in μm). The resulting samples were then exposed to enzymes (alpha-amylase and glucoamylase) to convert corn starch into glucose. A comparison of scanning electron micrographs of raw and sonicated samples showed the development of micropores and the disruption of cell walls in corn mash. The corn particle size declined nearly 20-fold following ultrasonic treatment at high power settings. The glucose release rate from sonicated samples increased as much as 3-fold compared with the control group. The efficiency of ultrasound exceeded 100% in terms

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of energy gain from the sugar released over the ultrasonic energy supplied. Enzymatic activity was enhanced when the corn slurry was sonicated with simultaneous addition of enzymes. This finding suggests that the ultrasonic energy did not degrade or denature the enzymes during the pretreatment.

Keywords:

Corn slurry, dry corn milling, enzyme stability, ethanol yield, starch, glucose yield, ultrasonic pretreatment, particle sizes

Introduction

The dependence on nonrenewable fossil fuel reserves to maintain quality of life, to sustain economic vitality, and to power the vast transportation networks is one of the most important problems facing the world today. Dwindling reserves in the face of rapidly increasing demands, combined with the environmental devastation resulting from global warming and acid deposition, demand the development of a sustainable, affordable, and environmentally friendly energy source. Ethanol is such a renewable clean fuel and is mainly produced from corn in the United States. Ethanol production in the United States is expected to grow to 7.5 billion gallons by 2012 from the current 4.5 billion gallons [1,2]. In the dry milling operation, the corn is hammer-milled, mashed, cooked, and treated with enzymes to break down the starch to produce fermentable sugar. The released sugar is fermented to ethanol by yeasts, which are recovered by distillation and dewatered using molecular sieves.

There has been much debate on the net energy gain from the conversion of corn starch to ethanol [3,4]. Farrell et al. [2] claimed that the net energy losses reported in the literature was due to the omission of co-products and the use of some obsolete data in the calculation. There is also the possibility of improving the economics of dry-grind corn milling plants by process improvements. For example, shortening of liquefaction and fermentation times, lowering the enzyme dosages [5], improving the overall starch hydrolysis, and eliminating some of the unit processes could reduce the production cost. The use of ultrasonic technology could provide a practical solution to improve ethanol yield at a lower cost by addressing all of these possible improvements.

Ultrasound defines sound waves at a frequency above the normal top of the hearing range of humans (> 15-20 kHz). When the ultrasound wave propagates in a medium such as a liquid or slurry, it produces cavitation [6,7] and acoustic streaming [8]. The cavitation generates powerful hydromechanical shear forces in the bulk liquid [9], which disintegrate nearby particles by extreme shear forces. The main benefit of streaming in corn slurry processing is mixing, which facilitates the uniform distribution of ultrasound energy within the slurry mass, convection of the liquid, and dissipation of any heating that occurs.

Ultrasonication has been applied widely in various biological and chemical processes. The use of high power ultrasound treatment enhanced starch-protein separation in a wet milling operation [10]. Li et al. [11] employed ultrasound to enhance oil extraction from soybeans. The authors reported an 11.2% increase in oil yield following 3 hours of sonication at an ultrasonic intensity of 47.6 W/cm^2. Ebringerová et al. [12] employed ultrasound to improve the extraction of active xylan and heteroxylan from corncobs and corn hulls, respectively. Wood et al. [13] studied the effects of ultrasonic treatment on ethanol fermentation from mixed office paper. The authors demonstrated that sonication of recycled paper increased ethanol production by as much as 20%. However, the use of high-power ultrasound in dry grind corn milling is a new concept. Since the release of starch from corn and subsequent conversion of starch to fermentable sugar is an enzyme-mediated reaction, reduction in corn particle size and efficient mass transfer of enzyme to substrate are the keys to enhanced enzyme activity. Ultrasound may also degrade enzymes due to localized heat, sonochemical reaction, and intense shear, if enzymes are added prior to sonication [14]. Sonication conditions, e.g., ultrasonic density (W/ml), sonication time, amplitude, and types of enzymes govern the enzyme stability. Such information is not readily available in the literature.

The use of high-power ultrasound has the potential to reduce the corn particle size and to free lipid-bound starch due to acoustic cavitation. In addition, it also facilitates better mass transfer of enzymes due to intense mixing resulting from acoustic streaming. Thus, the integration of an ultrasonic unit prior to liquefaction and saccharification could enhance the overall sugar release from corn for subsequent fermentation to ethanol. Based on these premises, the objectives of this preliminary investigation were to:

(i) Examine the efficiency of a bench-scale ultrasonic system to disintegrate the raw and cooked corn slurry mashes at different power inputs;

(ii) Investigate the effectiveness of ultrasound in releasing fermentable sugar from raw and cooked corn mashes at different power inputs; and

(iii) Study the effects of ultrasound on enzyme stability.

Experimental Section

Corn Samples and Enzymes Preparation

Corn slurry samples, raw and cooked, were obtained from Midwest Grain Processors (MGP) – a dry-grind corn ethanol plant in Lakota, IA – in a chilled container on a weekly basis. Milled corn (ground corn) was obtained from Lincolnway Energy (LE) – a dry-grind corn ethanol plant in Nevada, IA – and was mixed with double distilled water to prepare mash before sonication. The corn slurry sample from MGP contained a suboptimal amount of alpha-amylase and was stored at 4°C prior to use to reduce the enzyme activity. Two types of enzymes were studied, namely STARGEN^{TM} 001 (456 granular starch hydrolyzing units (GSHU)/g) obtained from Genencor International (Palo Alto, CA) and *amyloglucosidase* (300 units/ml) from *Aspergillus niger* (Sigma-Aldrich, St. Louis, MO). The STARGEN^{TM} 001 enzyme was used in raw corn slurry samples from MGP and corn mash samples from LE, while glucoamylase was used only in cooked corn slurry samples obtained from MGP. The STARGEN^{TM} 001 enzyme contains *Aspergillus kawachi* alpha-amylase expressed in *Trichoderma reesei* and a glucoamylase from *Aspergillus niger* that functions synergistically to hydrolyze starch into glucose.

Ultrasonic Pretreatment and Incubation

Raw and cooked corn slurry samples were sonicated using a Branson 2000 Series bench-scale ultrasonic unit for 20 and 40 seconds. The system has a maximum power output of 2.2 kW and operates at a frequency of 20 kHz. Sonication tests were carried out at three different amplitude (power) levels: low, medium, and high. Initial sonication tests were conducted using 10 ml of corn slurry sample mixed with 25 ml of acetate buffer (to maintain a pH of 4.3) and 0.05 % (v/v) of enzyme in a 50 ml polypropylene centrifuge tube. Enzymes were added to corn slurry samples after sonication (SA) or prior to sonication (SD). The control samples were not subjected to sonication. The experimental conditions are summarized in Table 3.1. After sonication, samples were incubated for 3 hours in a rotary shaker at 100 rpm and 32°C. All tests were conducted in triplicate with different batches of corn slurry samples.

The reported power levels in the table are the average values during sonication based on the initial and final power levels indicated by the power supply system. The static power, so-called air power (P_{air}), was subtracted from these values prior to energy calculation. The static power is the power required to run the system in an unloaded condition (in air). The power levels were varied by varying the amplitude at the horn tip through pulse width modulation voltage regulation to the converter. The horn was a standard 20 kHz half-wavelength catenoidal titanium with a flat 13 mm diameter face (gain = 1:7.5). The amplitude at the tip of the horn was approximately 180, 240, and 299 μm_{pp} at low, medium, and high power levels, respectively. It is important to note that these amplitudes are higher than typically used in industry, to examine a wide range of experimental values.

Table 3.1 Experimental Conditions

Sample source	Midwest Grain Processors (MGP)	Lincolnway Energy (LE)
Sample description	Corn slurry: Milled corn + water and partial amount of alpha-amylase: → Raw → Cooked	→ Milled corn
Sample preparation	None	Mix with water
Final sample prior to pretreatment	→ Raw corn slurry + α-amylase → Cooked corn slurry + α-amylase	→Raw corn slurry
	Full scale process samples	Laboratory prepared samples

Sample nomenclatures	Control	SA_{20}/SA_{40}	SD_{20}/SD_{40}
Dissipated power	0 Watt	Low : 274 ±5 Watt Medium : 350 ±5 Watt High : 475 ±15 Watt	
Experiments	Raw or cooked corn slurry with and without enzymes	Raw or cooked corn slurry with enzymes addition after sonication for 20 or 40 seconds	Raw/cooked corn slurry with enzymes addition prior to sonication for 20 or 40 seconds

Sonication Chamber Design

The sonication tests conducted with polypropylene centrifuge tubes showed a significant increase in the temperature of the corn slurry due to limited heat loss through a small surface area to volume ratio and the inherent low thermal conductivity of the base material (~ 0.34 W/m.K) [15]. In addition, there appeared to be significant attenuation of energy within the plastic tube itself. Thus, additional experiments were conducted with a specially designed glass sonication chamber, as shown in Figure 3.1, to examine the effect of temperature on glucose yield. The reaction chamber had a higher surface area to volume ratio and high thermal conductivity (~0.8 W/m.K) [16]. During the experiments, the temperature of the corn slurry was measured before and after ultrasound treatment in order to calculate the temperature increase (Δθ) during sonication. The temperature of the inlet water was approximately 10°C.

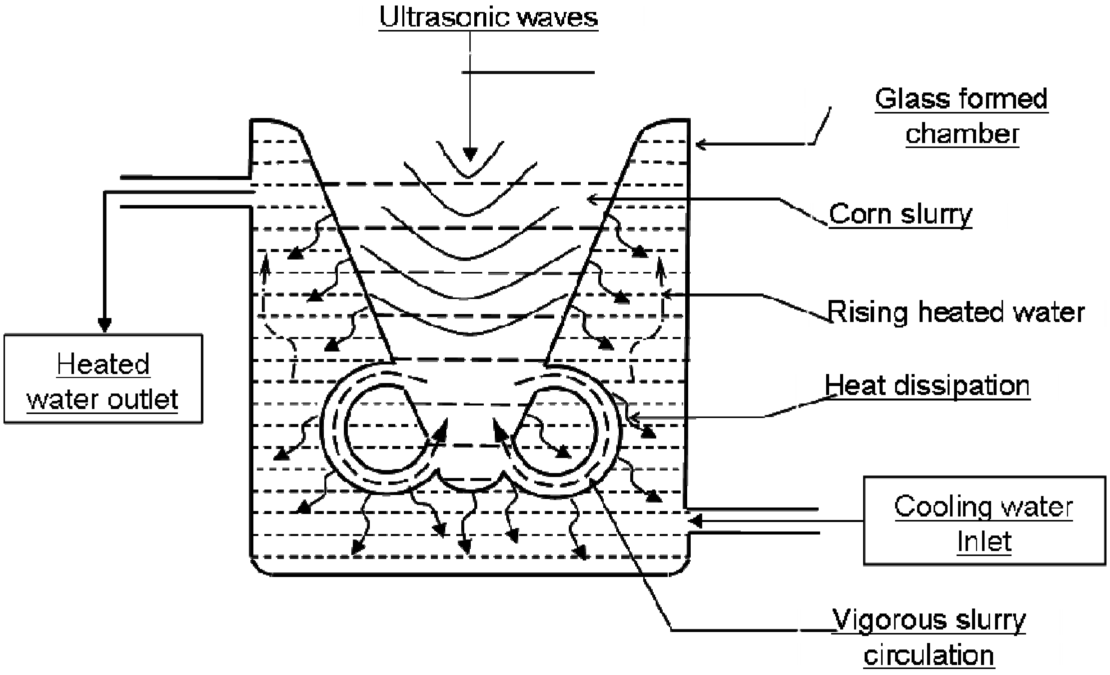


Figure 3.1 Schematic of Glass Sonication Chamber

Analytical Procedures

After sonication, 10% (v/v) 4M HCl Tris buffer (pH 7.0) was added to the samples to terminate the enzyme activity. Samples were centrifuged at 10,000 g for 20 minutes and then sieved through a U.S. standard mesh number 200 screen. Supernatant was then analyzed for glucose concentration using a modified dinitrosalicylic acid (DNS) method [17]. A sample size of 100 µl was removed from the batch (~35 ml), then mixed thoroughly with 1 ml of DNS reagent. The DNS reagent consisted of 0.25 g of 3,5 dinitrosalicylic acid, 75 g sodium potassium tartrate, 50 ml of 2M NaOH, and distilled water up to 250 ml. The solution was heated to 100°C for 10 minutes and then cooled down in an ice bath. The absorbance of the sample was measured at a wavelength of 570 nm using a spectrophotometer (ThermoSpectronic Genesys 2 – model W1APP11, Rochester, NY). Glucose concentrations were calculated from the calibration curve obtained using absorbance data for standard solutions of D-glucose reacted with DNS reagent prepared as above.

The particle size distribution of the corn slurry before and after ultrasonic treatment at different power levels was determined using a Malvern particle size analyzer (Mastersizer 2000, Malvern Inc., Worcestershire, United Kingdom). In addition, scanning electron microscopy (SEM) examination of the corn slurry samples was carried out. Prior to SEM examination, the samples were fixed with 3% glutaraldehyde (w/v) and 2% paraformaldehyde (w/v) in 0.1M cacodylate buffer (pH 7.2) for 48 hours at 4°C. Samples were rinsed three times in this buffer and post-fixed in 1% osmium tetroxide for 1 hour, followed by two 5-minute washes in buffer. The samples were then dehydrated in a graded ethanol series up to 100% ultrapure ethanol followed by substitution into hexamethyldisilazane and allowed to air dry. When dried, the samples were placed onto carbon adhesive coated aluminum stubs, sputter coated (Denton Desk II sputter coater, Denton Vacuum, LLC, Moorestown, NJ) with palladium/gold alloy (60/40), and imaged using a JEOL 5800LV SEM (Japan Electron Optics Laboratory, Peabody, MA) at 10 kV with a SIS ADDA II for digital image capture (Soft Imaging Systems Inc., Lakewood, CO).

Energy Dose Calculations

Ultrasonic dose is defined as the amount of energy supplied per unit volume of corn slurry and is expressed as Ws/ml or kWs/ml (J/ml or kJ/ml) [$ML^{-1}T^{-2}$]. Assuming the solids content of the samples remains relatively constant, the ultrasound dose is a practical method of expressing the energy input for the disintegration of corn slurry on a volumetric basis. The ultrasonic dose was estimated using the following relationship:

$$P_{avg} = \left(\frac{P_{initial} + P_{final}}{2} \right) - P_{air}$$

$$Q_{avg} = \frac{P_{avg}}{V}$$

$$E\text{ - }Density_{avg} = Q_{avg} \cdot t$$

Where : **Eq. 3.1**

P = Power

Q = Average power density (W/ml)

V = Volume of sample (ml)

t = Sonication time (second)

$E\text{ - }Density$ = Energy density (J/ml)

The total energy dissipated (E_{in}) into each sample was calculated based on the average power and sonication time:

$$E_{in} = \int_{t_0}^{t_f} P dt \sim E_{in} = P_{avg} t \qquad \textbf{Eq. 3.2}$$

Where t_0 and t_f are the initial and final times during sonication.

The total energy delivered during sonication (energy out, E_{out}) was calculated based on the chemical energy of the additional glucose produced compared with the control. The change of glucose mass yield compared with the control was calculated, and the energy of the glucose was estimated by assuming a conservative energy density of 15,740 kJ/kg for glucose if fully oxidized [3]. The overall efficiency *(Eff)* of sonication was calculated using the following equation:

$$Eff = \frac{E_{out} - E_{in}}{E_{in}} x100\% \qquad \textbf{Eq. 3.3}$$

Results and Discussion

Scanning Electron Microscopy Examination

The scanning electron micrographs (SEM) of raw and cooked corn slurry samples before and after sonication at high power levels are shown in Figure 3.2. The figures (Figure 3.2, A and C) display cells that appeared almost fully intact, and there were starch granules confined within the cells. With ultrasonic treatment for 40 seconds, nearly complete disintegration of cells was observed, with large numbers of fragmented cell materials (Figure 3.2, B and D). Several micropores were also visible within the disintegrated corn particles. It is important to note that there is a 5-fold magnification difference between the Figure 3.2, C and D. At the same magnification, the treated sample would appear as indistinguishable particles. Thus, the SEM images demonstrated the changes in the structures of corn particles after ultrasound treatment.

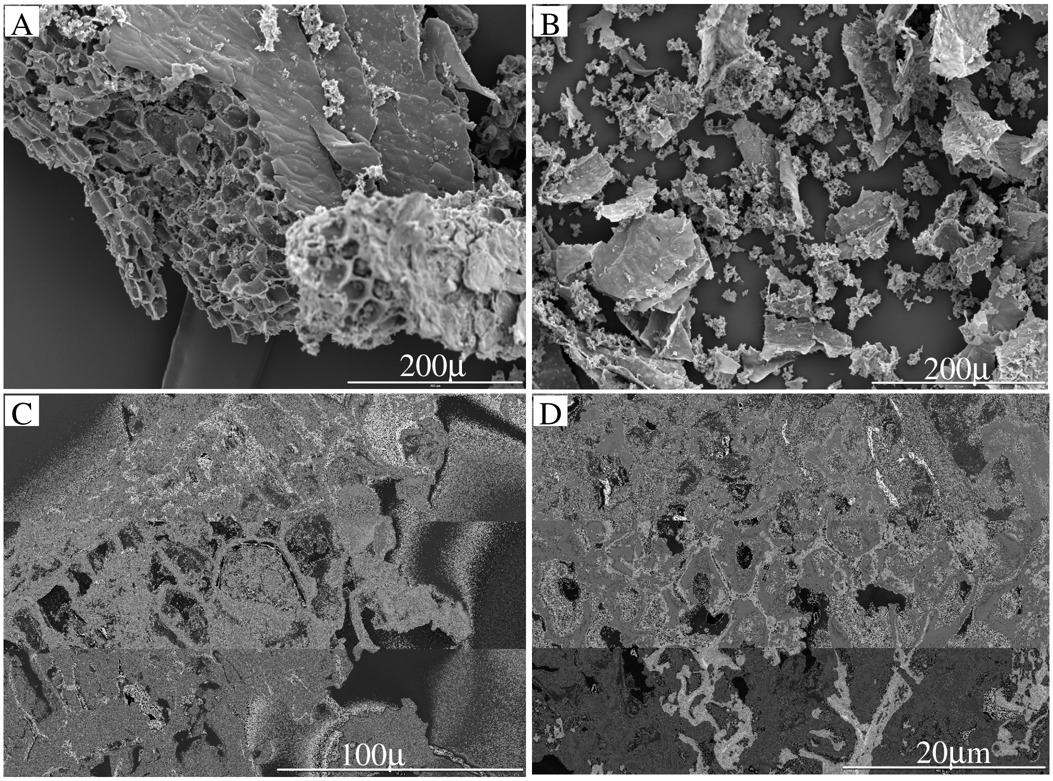


Figure 3.2 SEM Images of Corn Slurry: (A) Raw Corn (Control); (B) Raw Corn Sonicated (40 s); (C) Cooked Corn (Control); (D) Cooked Corn Sonicated (40 s)

Particle Size Distribution

The effect of ultrasonic treatment on corn particles was examined by sonicating both cooked and raw corn slurry samples, and the resulting particle size was compared with nonsonicated samples (controls). As seen in Figure 3.3C, the peak of the particle size distribution curve shifted from 800 µm to around 80 µm following sonication at high power levels for cooked corn slurry samples. In addition, the particle size reduction was directly related to power level and sonication time. The particle size reduction at the higher power level and longer sonication time is in close agreement with glucose yield under similar conditions, as discussed later. It is interesting to note that in all experimental sets, there were three inflection points at 50, 120, and 800 μm. The original inflection point (800 μm) is expected, since this is a function of the corn milling operation and represents particles that are not affected by the ultrasonication. It is believed that the second and the third inflection points corresponded to cell morphologies of the corn. For example, the 50 μm particles were probably individual starch grains, and the 120 μm particles were most likely clusters of these grains as well as residual cell debris. Similar results were also obtained for raw corn slurry (data not shown here).

Glucose Release

The concentrations of released glucose from raw corn slurry samples are presented in Figure 3.4A. Because all the experiments were conducted in batch mode, the comparisons were only made in relation to the control batch in order to eliminate errors associated with continuous enzymatic reactions. For example, the glucose release in the lower power group tests was compared with the control of the lower power group for each experiment. The highest glucose release increases of 32 and 27% with respect to the control group were obtained for SD_{40} at both low and medium power inputs, respectively. The glucose release, however, dropped by nearly 22% at the high power setting for SD_{40}. A similar trend was also observed for SD_{20}, in which the glucose release dropped by 11%. These tests were conducted without temperature control, and the final temperature of the treated samples increased in direct proportion to the power setting and treatment time. Therefore, these decreases in glucose concentration were attributed to excessive ultrasonic treatment, which resulted in degradation and denaturing of the enzymes during sonication at high power settings. This finding is in close agreement with glucose release data without enzymes (SA_{20} and SA_{40}), which showed an improvement in glucose release following sonication irrespective of power settings. The finding further suggests that the high power setting did not cause the gelatinization of starch due to sonication. Thus, to maximize glucose release, both sonication time and power level need to be optimized. The additional sugar yield from ultrasound treated samples could also be due to the release of starch that was bound to lipids and did not have access to the hydrolyzing enzyme.

Figure 3.4B shows the concentrations of glucose release for the cooked corn slurry samples. Because the enzyme alpha-amylase had already been added to the corn slurry during cooking, only *glucoamylase* was added during the sonication test. In these studies, the highest increase in glucose concentration (30%) with respect to the control group was obtained for SD_{40} at low and medium power inputs. The findings were in close agreement with those for raw corn samples (Figure 3.4A). The glucose release, however, dropped nearly 60% at high power levels. Interestingly, the SD_{20} group at high power setting had an increase in glucose concentration of 23%, which was not observed in the raw corn slurry experiments. This observation was not clearly understood.

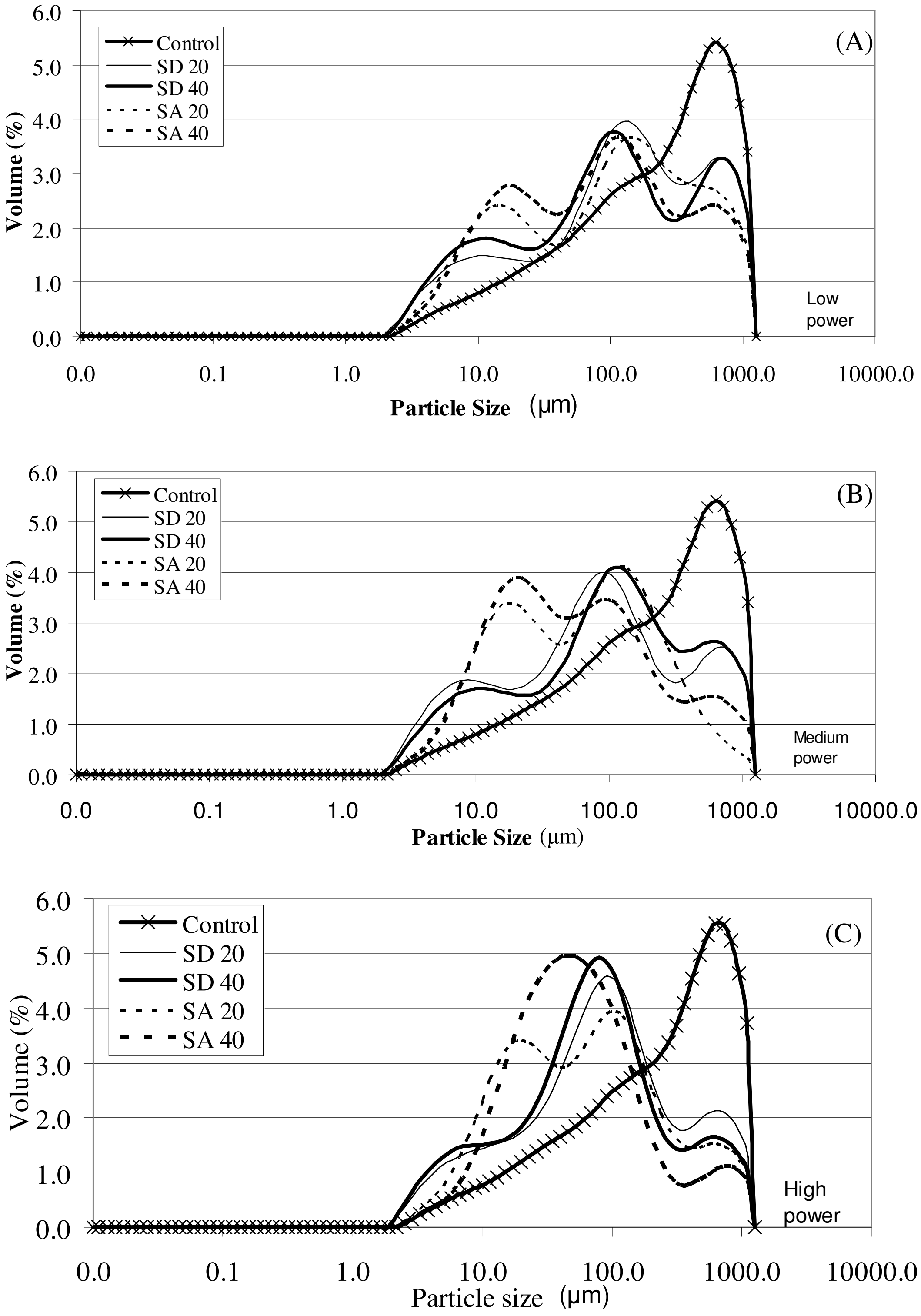
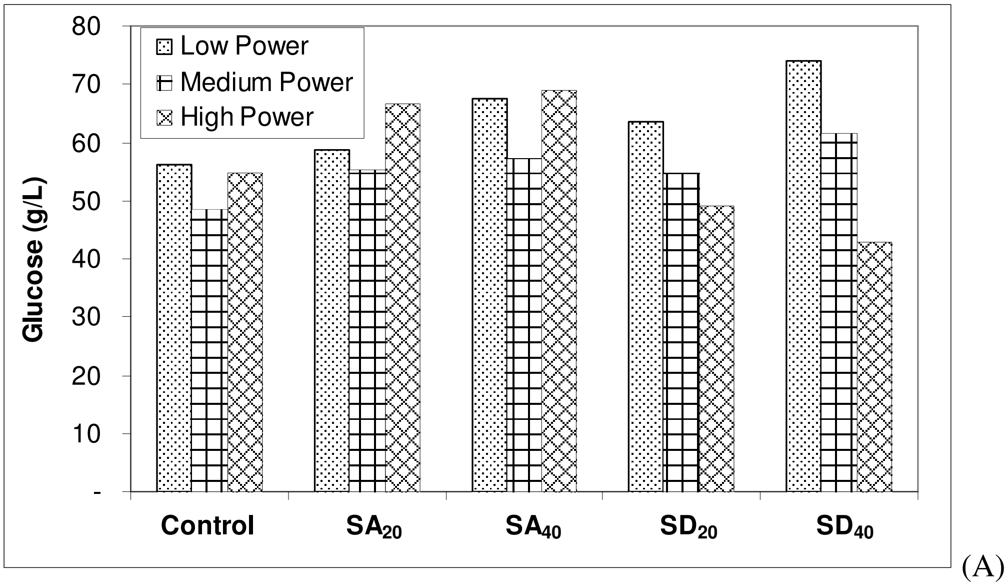


Figure 3.3 Particle Size Distribution (μm) of Cooked Corn Slurry for (A) Low Power, (B) Medium Power, and (C) High Power



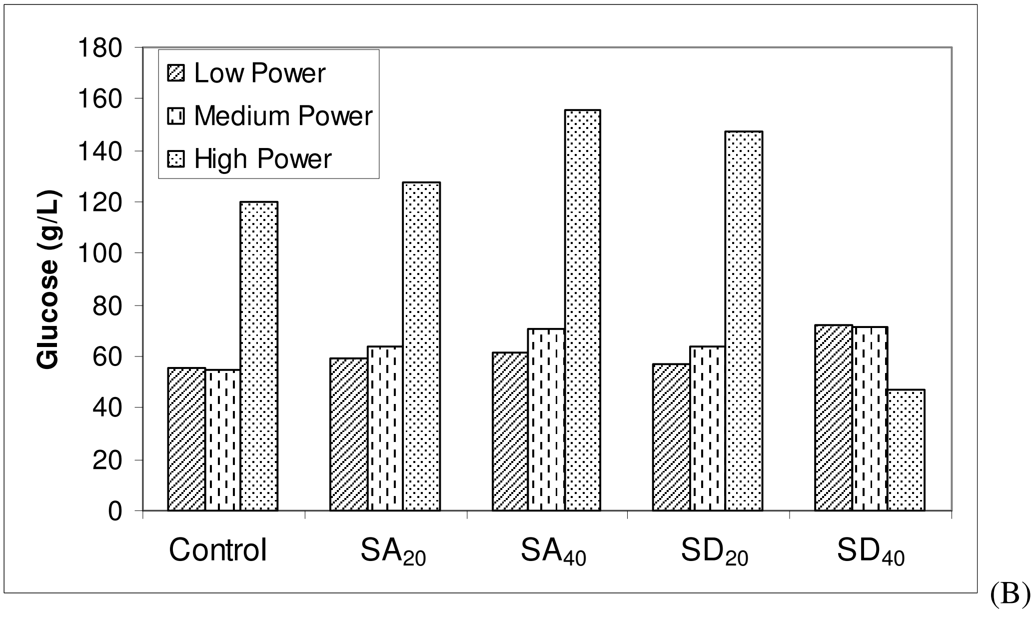


Figure 3.4 Glucose Release of (A) Raw and (B) Cooked Corn Slurry at Varying Power Levels

In order to characterize the effect of sonication on the rate of glucose release, corn slurry samples were treated at the medium power level (amplitude of 80 μm_{pp}) for 40 seconds. The samples were saccharified using the protocol elucidated earlier for up to 8 hours, and samples were collected at 1-hour intervals. In these experiments, a milled corn sample obtained from LE was used. The data was then fitted with standard reaction rate kinetics of Arrhenius form:

$$G(t) = G_{\infty}\left(1 - e^{-tk}\right) \qquad \textbf{Eq. 3.4}$$

Where $G(t)$ is the glucose concentration as a function of time (t), G_{∞} is the glucose concentration at time infinity, and k is the reaction rate coefficient. As seen in Figure 3.5, the reaction rate (k) for the control group (nonsonicated) was 3-fold higher than for the ultrasonic treated sample. This is because the final glucose concentration was much higher for the ultrasonic treated samples, thus requiring more time to reach the final reaction time. Based on this equation, the glucose concentration at completion of reaction for the sonicated sample would theoretically be 145 g/L compared to only 31 g/L for the controls, i.e., about 5-fold higher. It is believed that this significant enhancement of glucose release was due to the fact that no enzymes were introduced into the experiment until the final saccharification step. In the previous experiments, diffusion of the enzymes into the corn particles was not completely eliminated. Thus, during incubation, these enzymes became active and reduced the overall glucose release.

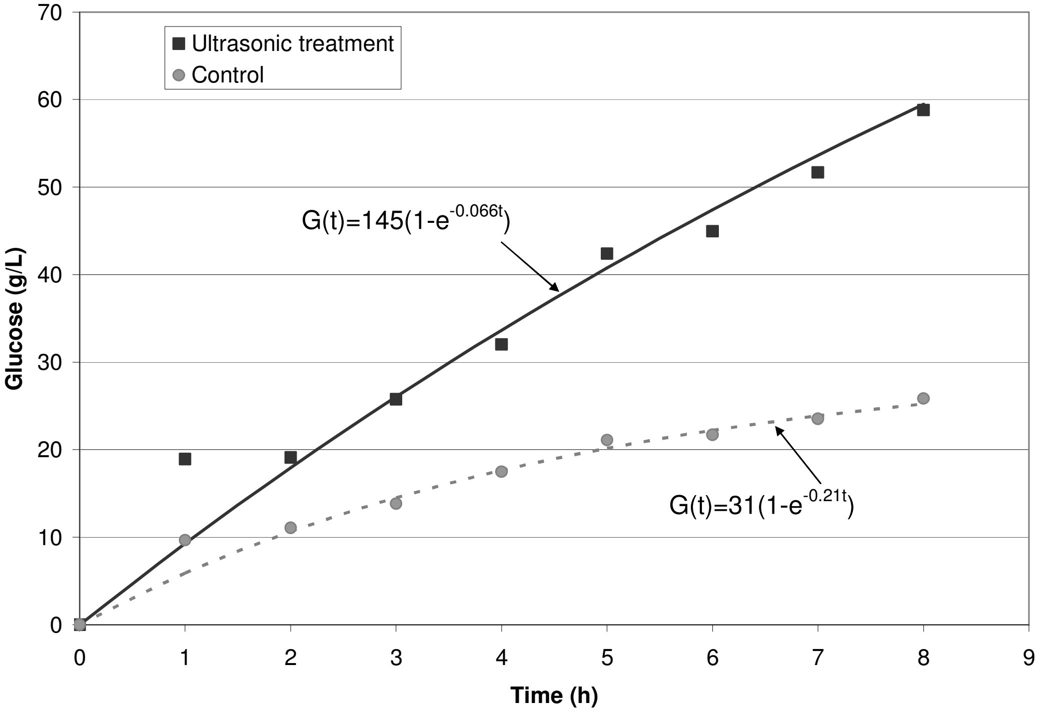


Figure 3.5 Glucose Release from Raw Corn Slurry at Different Incubation Times

Temperature Controlled Sonication Experiments

Based on preliminary experiments, the dissipated power was not consistently proportional to the set amplitude. The dissipated power (for 35 ml water load) in the glass chamber is shown in Figure 3.6. As is apparent from the figure, at amplitudes below 65 μm_{pp}, the power was proportional to amplitude; however, an inflection point was observed above this value. This could be due to excessive motion/agitation of the water that may have caused decoupling between the water and the horn. Such decoupling could reduce the transmission of energy/power from the horn to the water. Further increase of the amplitude continued to increase the power dissipation – as expected. This is an important observation when interpreting the results of the glucose release using the glass sonication chamber, as the power and amplitude were not continuously proportional over the ranges studied.

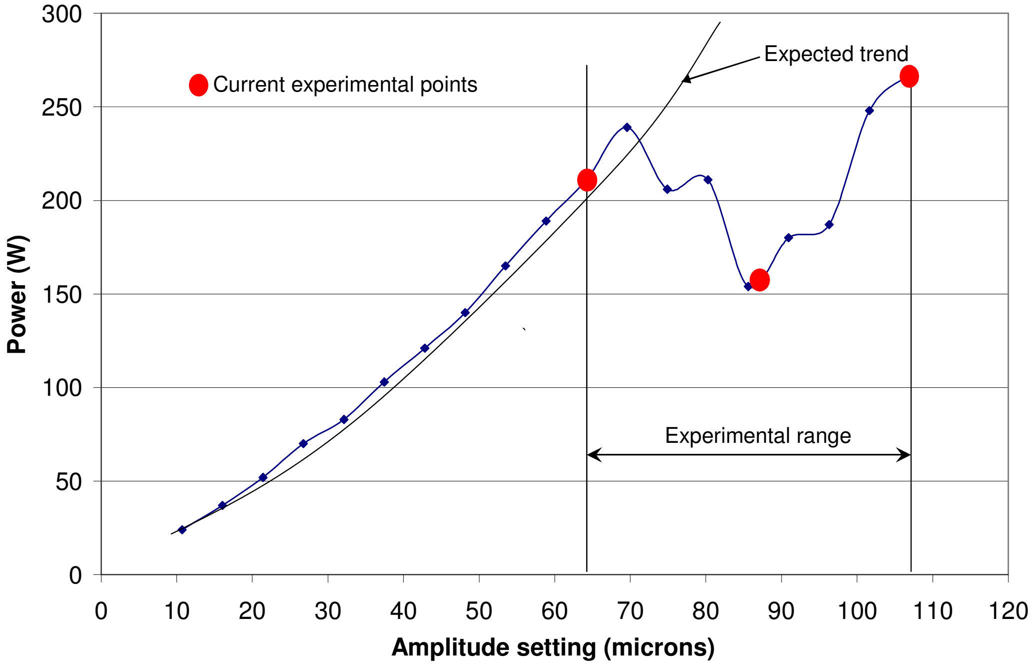


Figure 3.6 Power Dissipation in the Glass Chamber at Different Amplitudes

The relative glucose release at various power settings for the cooked corn slurry with the glass sonication chamber is shown in Figure 3.7. The relative glucose release was as high as 27% with a temperature increase (Δθ) of less than 5°C during 40 seconds of sonication at the lower power input. Thus, it is believed that the increase in glucose release of sonicated samples was not attributed to the thermal effect, but was related to particle size reduction, better mixing due to streaming effects, and the release of additional lipid-bound starch. As expected, the relative temperature increase was in direct proportion to power input and sonication time. It is important to point out that the power dissipation (as indicated by an internal power meter) in the glass sonication chamber was

50 to 75% higher than that in the plastic centrifuge tube for all experiments. It is believed that the chamber geometry as well as the mechanical impedance of the base material may have contributed to various levels of attenuation. The sugar release did not improve for corn slurry samples with prior enzyme addition at the higher amplitude. This again suggests the possibility of enzyme denaturation at higher power input. Thus, low power inputs appeared to be ideal for simultaneous sonication, liquefaction, and saccharification of corn slurry.

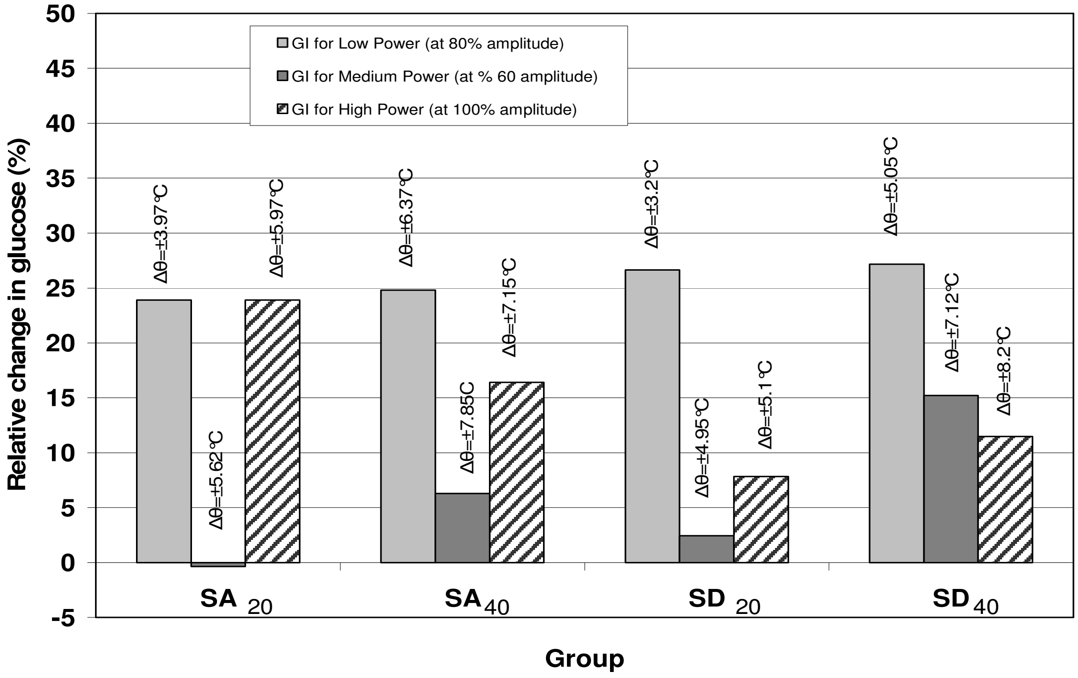


Figure 3.7 Relative Change in Glucose Concentration at High Power Setting with Glass Sonication Chamber (Cooked Corn Slurry)

Energy Balance

As seen in Figure 3.8, the overall efficiency of the glucose release from raw corn slurry ranged from 70 to 125%, depending on the treatment conditions. The efficiency greater than 100% indicates additional chemical energy from the release of extra sugar following sonication. At shorter treatment times, the efficiency was, however, better compared with higher power settings and longer treatment times. It is important to note that the trends were not dependent on the dissipated power (Figure 3.6), which may explain the relatively low efficiency at the medium power setting. While the efficiency shown in Figure 3.8 was directly correlated to the actual power, there currently is no explanation why the medium power setting resulted in such poor efficiency. This phenomenon was further confirmed by the lower power dissipation at medium power level (amplitude of 75 to 85 μm_{pp}) as shown in Figure 3.7.

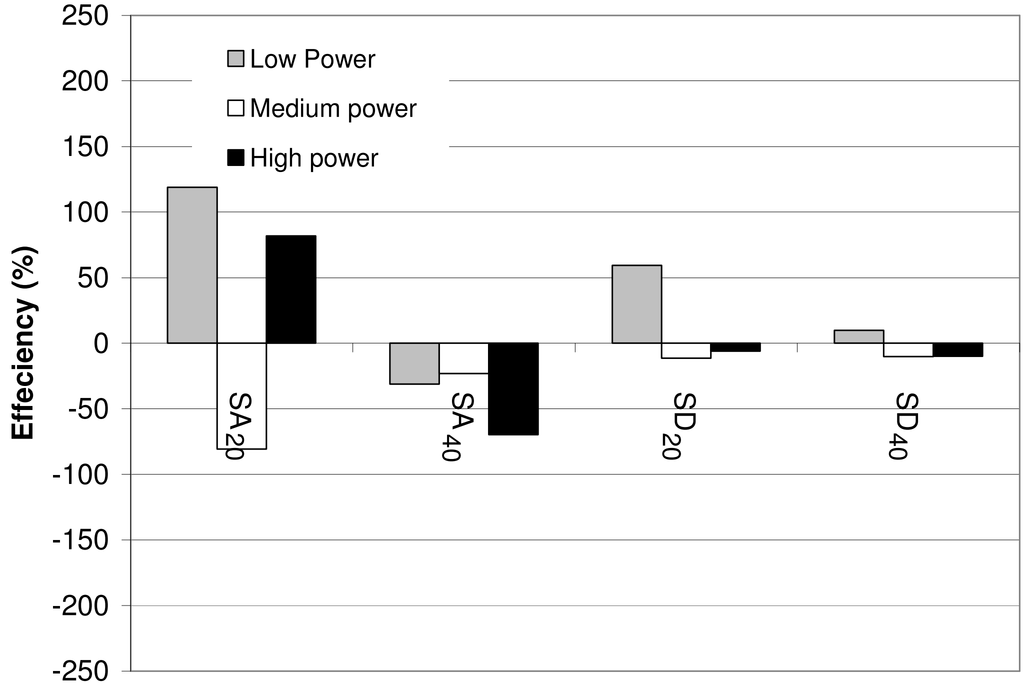


Figure 3.8 Sonication Efficiency for Raw Corn Slurry

The data suggest that there are economic benefits associated with the ultrasonication of corn slurry as measured by overall improvement in process efficiency. However, in order to fully appreciate the economic aspects of this technology, a cost-benefit model would need to include the initial capital, operations, and maintenance costs. While it is not possible to build such a model based on the data presented here, some insight might be gained by considering preliminary data (not reported here) that suggests that corn slurry flow rate above 15 gal/min per standard continuous flow through horn (a so called “donut horn”) can increase the glucose yield by 15%. Thus, for a 50 million gallon/year plant, the capital costs for the number of horns required at such a flow rate would be slightly over \$ 0.86 million, which is less that 1% of the entire costs of a plant of that size. The power requirements of an ultrasonic system for a 50 million gallon/year plant would be 200 kWh, amounting to approximately 1,700,000 kWh/year, with an annual cost of approximately \$100,000. The overall increase in ethanol production for such a plant would be 7,500,000 gallons/year, which would result in an additional net income of \$3.75 million per year at estimated profit of \$0.50/gallon. The payback period would therefore be only months, even with substantial maintenance costs considered. It is important to note that the marginal costs for additional ethanol production is dependent on the sale price of ethanol, the cost of energy inputs, and the price of the corn. Because of the relatively high operating efficiency of the proposed system, the additional operating cost would also be minimal. However, feasibility tests need to be completed to accurately determine these projected costs as well as identify issues such as effects on downstream processing and the effect on the main by-product, distiller’s dried grains with solubles (DDGs) that this additional pretreatment might introduce.

Summary

Ultrasonic pretreatment of corn slurry resulted in a nearly 20-fold reduction in corn particle size. The enzyme addition during sonication yielded better glucose release than enzyme addition after ultrasound treatment. The glucose release of sonicated samples improved by 30% with respect to the control group. The increase in glucose release from ultrasound treated samples was mainly due to reduction in particle size, better mixing due to streaming effects, and release of additional lipid-bound starch. Ultrasound treatment at a high power setting for longer sonication times resulted in nearly 60% reduction in glucose release due to denaturing of enzymes. The rate of glucose release for sonicated corn samples was 3-fold higher than for the non-sonicated controls. Based on energy balance calculations, there was an increase of two Joules of sugar equivalent energy for every Joule sonication input. Thus, integration of high power ultrasonics into dry corn milling has the potential to significantly improve the ethanol yield and improve profitability. Further studies are currently underway to optimize ultrasonic conditions (power input, sonication time, solids level, etc.) for continuous treatment and to examine final ethanol yield of sonicated samples.

Acknowledgement

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CHAPTER 4: SONICATION OF SUGARY-2 CORN: A POTENTIAL PRETREATMENT TO ENHANCE SUGAR RELEASE

A paper submitted to *Bioresource Technology*

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Abstract

The effects of high-powered ultrasonics on the conversion of sugary-2 maize (*Zea Mays L.*) to fermentable sugars were studied in this research. Ground sugary-2 maize mash was sonicated at 20 kHz and varying amplitudes (192-320$\mu m_{\text{peak-to-peak}}$) for 5, 10, 15, 20 and 40 s. Stargen^{TM} 001 enzyme, which contained both α-amylase and gluco-amylase, was added to the samples following sonication to hydrolyze the starch into fermentable sugars. There was a 3-fold increase in sugar conversion rate of the sonicated samples in comparison with the control (unsonicated) samples. The ultrasonic relative net energy gain in the majority of the experimental design space was greater than 1.0. This indicates that the released of stored energy (output energy) from additional sugar released was greater than the dissipated ultrasonic energy (input energy), thus making ultrasonics an efficient treatment. Scanning electron microscopy (SEM) pictures revealed that the

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sugary starch was partially gelatinized during sonication. This observation was confirmed by polarized-light microscopic images, where a deformed “Maltese cross” was found. Swelling power for samples sonicated at 40s reached 5.0 g/g while samples treated in conventional heating reached 4.0 g/g at 4 minutes treatment. It was also found that swelling power in the ultrasonicated sample initiated as quickly as 5s and increased rapidly. These results are evident that ultrasonics can enhance swelling and gelatinization compared to conventional heating. As the saccharification time increased, a model was formulated to fit the sugar release curve. The findings indicated that there was a significant effect on enzymatic activity when enzymes were added to the sample during sonication. Additionally, jet cooking and ultrasonication obtained similar theoretical starch conversion results after 3 hours saccharification. Thus, it is evident that ultrasonication could be considered a potential alternative to jet cooking.

Keywords:

Sugary-2 maize, gelatinization, ultrasound pretreatment, ethanol, glucose, energy efficiency, enzyme, corn

Introduction

Ethanol production is growing rapidly, resulting in a corresponding increase in research to develop new technologies to increase output and efficiency. Corn has been the main source of fermentable sugars for ethanol production in the United States. A number of study have explored corn mutants that have altered starch composition in the endosperm [1,2,3]. Sugary maize, commonly known as sweet corn, has been characterized to have a lower starch content but higher sucrose composition [4]. Takeda and Preiss [5] reported that sugary (B90) starch contained 30.6% amylose while normal (W64A) starch contained 18.5%. It is hypothesized that due to the higher amylose content, sugary maize could easily be hydrolyzed upon pretreatment. Additionally, sugary maize starch had lower gelatinization temperature than starch from normal corn and was considered suitable for application in starch-thinned acidic foodstuffs [6]. Since most studies on sugary maize applications have been limited to food application, the novelty of this research is that it focuses on potential of sugary corn for ethanol production and the utilization of ultrasonic energy to enhance sugar production from sugary corn.

Ultrasound defines sound waves at a frequency above the top of the normal human hearing range (>18-20 kHz). As ultrasound waves propagate in a liquid medium, cavitation occurs [7,8]. “Cavitation is the production of microbubbles in liquid when a large negative pressure is applied to it” [9]. The microbubbles are formed in the liquid media by acoustic forces [10]. Because of surface tension and the presence of other bubbles, foreign bodies, and gradients in the pressure waves, each bubble becomes unstable beyond a critical size and eventually collapses violently [9]. As the bubbles collapse, localized temperatures reaching up to 5000°K and pressures up to 180 MPa are achieved [11,8]. Ultrasound waves in liquid media also produce acoustic streaming, which is used to facilitate the uniform distribution of ultrasound energy in the media, convection of the liquid and dissipation of any heat produced [12,8].

Ultrasonics has been widely used in various biological and chemical applications. Zhang, et al. [13] reported the use of ultrasonic treatment to enhance protein-starch separation for use in the wet-milling industry. Ultrasonics has also been employed to assist the extraction of resveratrol from grapes [14]. Wang [15] utilized ultrasound treatment to extract protein from autoclaved flakes soybena, alcohol washed flakes soybean and commercial soybean. Wood et al. [16] have studied ultrasonics to enhance ethanol yield from simultaneous saccharification and fermentation of mixed office paper. They achieved a 20% increase in ethanol yield from their sonicated samples.

Khanal et al. [17] have applied ultrasound to break down the particle size of milled commodity corn for subsequent improvement in sugar released in corn dry-milling. The authors reported a 3-fold increase in sugar production rate from the sonicated corn slurry. Motivated by the preceding success, this study examined the potential of exposing sugary corn slurry to high-power ultrasonics to enhance sugar production. Because sugary corn has lower crystallinity and a lower gelatinization temperature than normal corn [18], it was postulated that the starch in sugary corn would be easier to break down compared to normal maize under the same conditions of ultrasonication. Basing on this premise, the objectives of the study were to determine the efficacy of ultrasound treatment in releasing fermentable sugar in sugary corn and to compare sugar release of sonicated sugary corn to sonicated commodity corn.

Materials and Methods

Sample preparation and ultrasonics conditions

Corn slurry samples were prepared with a composition of 3 g of dry ground sugary corn (B90), 25 ml 0.1 M pH 4.5 acetate buffer, and 7 ml de-ionized water (DI). In order to compare this study with previous study [17], commodity corn (Lincolnway

Energy, Nevada, IA) was also used in this study. During the preliminary study, several screening tests were conducted varying the total solid concentration. It was found that at higher total solid concentration, the slurry solidified into a gel before the ultrasonication treatment had completed. That is because the ultrasonic treatment enhanced gelatinization, thus there was a self limiting level of solids. Therefore, 3g ground corn was used.

The enzyme used was STARGEN^{TM} 001 (456 granular starch hydrolyzing units in GSHU/g) from Genencor International (Palo Alto, CA), which contained *Aspergillus kawachi* α-amylase expressed in *Trichoderma reesei* and glucoamylase from *Aspergillus niger* that hydrolyzes starch dextrins into glucose. The composition of sugary corn was obtained using a Near-Infrared (NIR) Infratec^{TM} 1241 Grain Analyzer (FOSS Tecator, Eden Prairie, MN).

Corn slurry samples were sonicated using Branson 2000 Series (Branson Ultrasonics, Danbury, Connecticut, USA) bench-scale ultrasonic unit for 5, 10, 15, 20 and 40 s. The system operates at a maximum power output of 2.2 kW and a frequency of 20 kHz. The ultrasonic treatments were carried out in 50-ml polypropylene centrifuge tubes using three different amplitudes (power): low, medium and high (Table 4.1). The horn was a standard 20-kHz half-wavelength catenoidal titanium with a flat 13-mm diameter face (gain = 1:8). STARGEN^{TM} 001 enzymes (18 μl) were added after sonication. The samples were then incubated (liquefied and saccharified) for 3 hours in a rotary shaker at 150 rpm and 32°C. All experiments and analytical procedures were conducted in duplicate and triplicate, respectively.

Table 4.1 Average ultrasonic power dissipated at different amplitudes

Parameters	**Power levels**		
	Low	**Medium**	**High**
Average power dissipated (J/s)	164 - 174	205 - 237	248 - 330
Amplitude (μm_{pp})	192	256	320
Average power density (W/g)	56.3	73.7	96.3
Average power density (W/ml)	4.8	6.3	8.3
Average energy density (J/ml) (20 s sonication time)	96	126	166
Average energy density (J/ml) (40s sonication time)	192	252	332

Jet cooking

Jet cooking was performed in the Iowa State University Center for Crop Utilization Research (CCUR) Wet Pilot Plant, Ames, IA. Corn slurry samples at 30% solids were continuously stirred in the feed tank (Figure 4.1). The boiler steam pressure and temperature were 80 psi and 150°C, respectively. The corn slurry was pumped through the jet cooker by means of the steam pressure. In order to compare jet cooking with ultrasonics, similar saccharification procedure was followed. A 10-ml representative sample was taken, added with 25 ml 0.1 M pH 4.5 acetate buffer and 18 µl STARGEN^{TM} 001 enzyme. The samples were then incubated (liquefied and saccharified) for 3 hours in a rotary shaker at 150 rpm and 32°C.

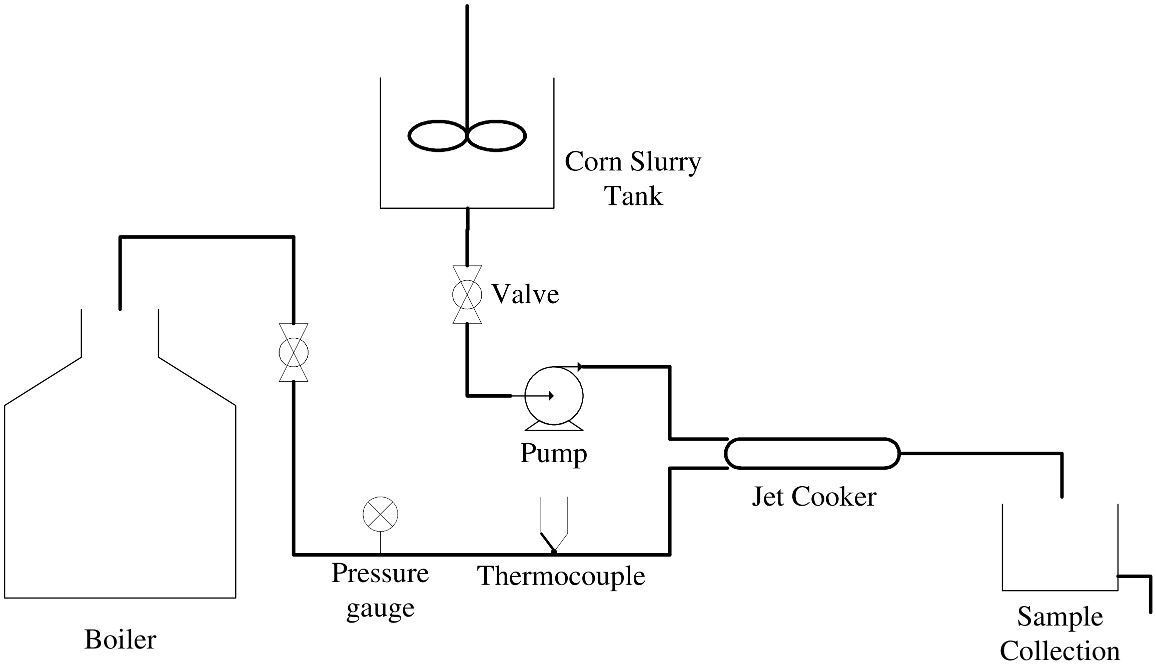


Figure 4.1 Jet cooking Experimental Set-up (Facility at Iowa State University Center for Crop Utilization Research Wet Milling Plant).

Analytical procedures

After liquification and saccharification, 2 ml of 4M HCl-Tris buffer (pH 7) were added to the samples to stop the enzymatic reaction at particular times. The slurry was then centrifuged at 10,000 rpm (12,096 x g) using a Beckman Coulter Avanti J-20xPI with Rotor JA25.5 (Fullerton, CA) for 10 min to separate the unsaccharified corn. The supernatant was then analyzed for reducing sugar using the modified dinitrosalicylic acid (DNS) method [19, 17].

A modified swelling power (g/g) and water solubility index (%) test were determined using the method proposed by Leach, et al. [20]. Three (3) g of dry corn was mixed with 32 ml water and sonicated at high power for 5, 10, 15, 20 and 40s. After sonication, the corn slurry was centrifuged at 2,500 rpm for 15 minutes. Swelling power (SP) was calculated by dividing the sedimented paste by the corn dry weight while water solubility index (WSI) was obtained by dividing the solid content in the supernatant by the corn dry weight.

To compare ultrasonication with conventional heating, the temperature profile of the sonicated sample was measured during the ultrasonic treatment (Table 4.2). Swelling power and water solubility index test was conducted using conventional heating with similar temperature histories. Three (3) g of dry corn was heated in 32 ml water for 15, 120 and 240 seconds. The 32 ml water with out corn was initially heated to the corresponding ultrasonic treatment temperature before dry corn sample was added. In more detail, the temperature was varied corresponding to the final temperature of the sonicated samples (Table 4.2). The sample was then cooled in ice water bath and centrifuged at 2,500 rpm for 15 minutes. Swelling power was calculated from the sedimented paste and water solubility index was calculated from the supernatant.

Ultrasonic Energy Calculation

Ultrasonic energy dissipated is described as the amount of energy supplied per unit volume of corn slurry in W/ml. The equations below summarize the amount of ultrasonic energy dissipated per unit volume.

$$P_{avg} = \left(\frac{P_{initial} + P_{final}}{2} \right) - P_{air}$$

$$Q_{avg} = \frac{P_{avg}}{V}$$

$$E\text{ - }Density_{avg} = Q_{avg} \cdot t$$

Where : [Eq. 4.1]

P = Power

Q = Average power density (W/ml)

V = Volume of sample (ml)

t = Sonication time (s)

E - Energy density (J/ml)

Ultrasonic Relative Net Energy Gain

The energy gain was established by comparing the total energy delivered during sonication (Energy in, E_{in}) to the chemical energy of the additional sugar produced relative to the control group (Energy out, E_{out}). By assuming D-glucose as the standard monosaccharide used for reducing sugar analysis, and by further assuming the energy density of glucose (15,740 kJ/kg) [17], the total energy dissipated into the sample (E_{in}) and the overall ultrasonic energy gain can be described by Eqs. 4.2 and 4.3.

$$E_{in} = \int_{t_0}^{t_f} Pdt \sim E_{in} = P_{avg}t \qquad \text{[Eq. 4.2]}$$

$$Gain = \frac{E_{out} - E_{in}}{E_{in}} \qquad \text{[Eq. 4.3]}$$

Where t_0 and t_f are the initial and final sonication exposure expressed in seconds.

Microscopy

Selected wet corn slurry samples were viewed at 40x magnification with a scanning electron microscope (Hitachi S-2460N VP-SEM, Hitachi, Ibaraki, Japan). Optical pictures were taken with cross-polarized filters using a Nikon microscope (Labophot Nikon HFX-II, Japan).

Table 4.2 Temperature profile of ultrasonicated sugary corn slurry at high power

Sonication Time (s)	**Initial Temperature (°C)**	**Final Temperature (°C)**
5	20-22	25-27
10	20-22	32-35
15	20-22	42-45
20	20-22	50-52
40	20-22	68-70

Results and Discussion

Starch conversion at different sonication conditions

Because starch is primarily composed of D-glucose monomers, D-glucose was used as the standard reducing sugar assay in this study. The sugar concentration obtained was compared with the theoretical sugar yield based on the starch content of the sugary corn to obtain percentage sugar conversion (Figure 4.2). The composition of ground sugary corn was found to be: 12.2% protein, 68% starch; 4.3% oil and 15% moisture, with a bulk density of 1.28 g/ml (or 1277 kg/m^3). Because all experiments were conducted in batch mode, the comparisons were presented in relation to the control batch that did not undergo ultrasound pretreatment.

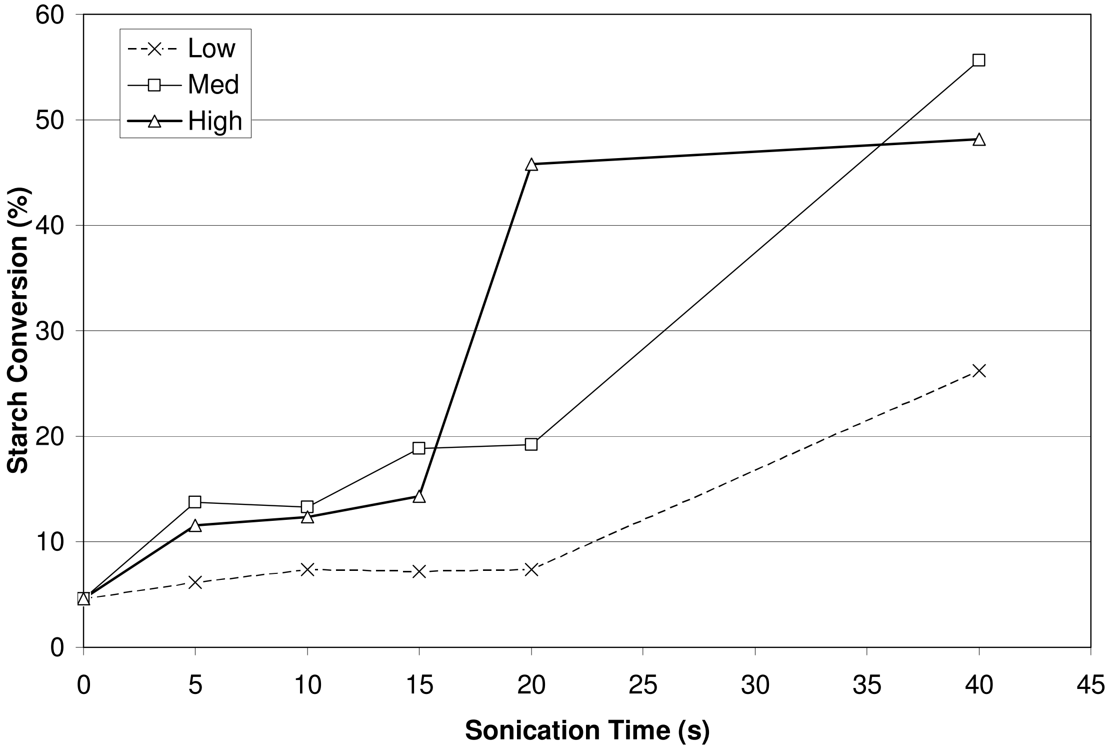


Figure 4.2 Percent starch conversion of sugary corn at varying sonication times & varying sonication power (low, medium, high) during a 3-h incubation period

Figure 4.2 shows sugary corn converted to reducing sugar as a function of sonication time at different power levels for an incubation time of 3 hours. A sonication time of 0 s indicates the control group. The samples subjected to ultrasonics gave a 5- to 6-fold increase in reducing sugar compared to the control group during the test period. Starch conversion ranged from 4% for the control to about 67% for the sonicated groups. The conversion of starch to sugar was proportional to time and power (amplitude) level. This is consistent with previous results [17], which were attributed to particle size reduction and streaming effects. At the low power level and the short treatment times, there was little effect on starch conversion. On the other hand, there was only marginal difference in the starch conversion of medium and high power levels. This could be attributed to the higher amylose content which was easily hydrolyzed during ultrasonic treatment.

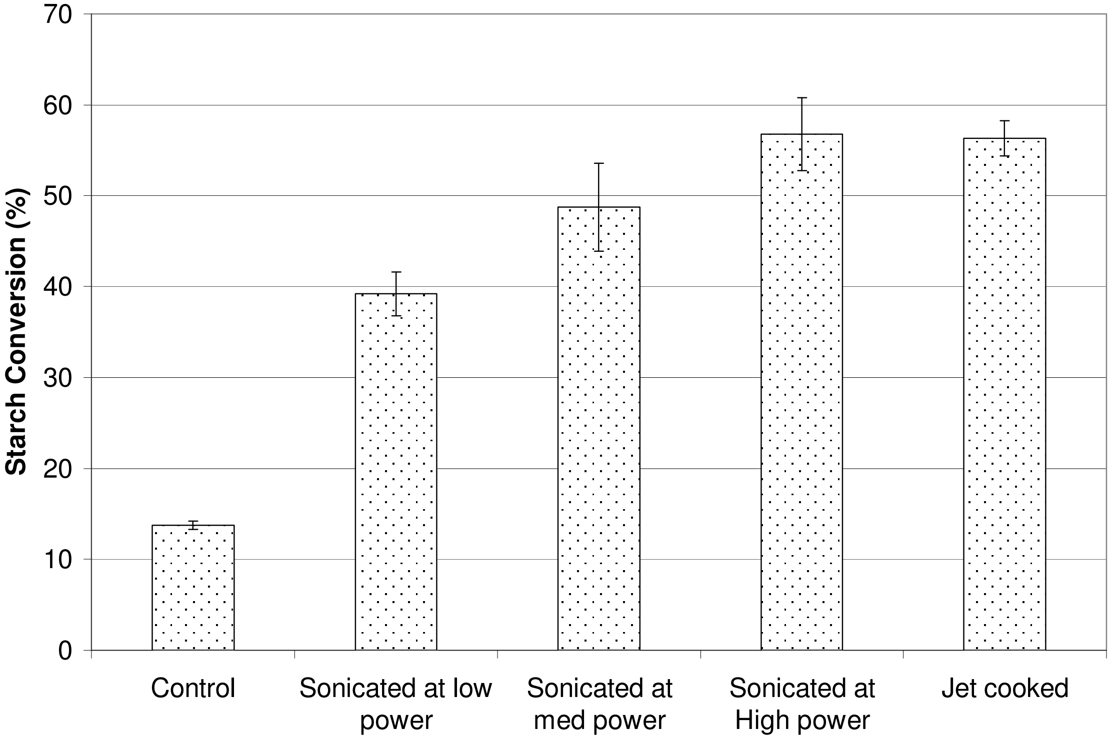


Figure 4.3 Theoretical starch conversion of sonicated and jet cooked sugary corn during a 3-h incubation period. ***(Ultrasonics conducted at 40 seconds sonication time)***

Figure 4.3 shows a comparison of saccharification yield between ultrasonics and jet cooking. For a 3 h liquefaction and saccharification period, the theoretical starch conversion within the experimental design ranged from 13-56%. It is important to note that starch conversion is relative to a theoretical starch conversion (100% conversion) under prolonged saccharification times. The untreated control group resulted in the lowest conversion at 13.7% while samples sonicated at low, medium and high power for 40 seconds resulted in 39.2%, 48.8%, 56.8%, respectively. The sugar released after saccharification was found to be proportional to the ultrasonic energy dissipated. The jet cooked sample also obtained similar starch conversion value as the sample sonicated at high power. This indicates that ultrasonics is comparable to jet cooking in releasing fermentable sugars.

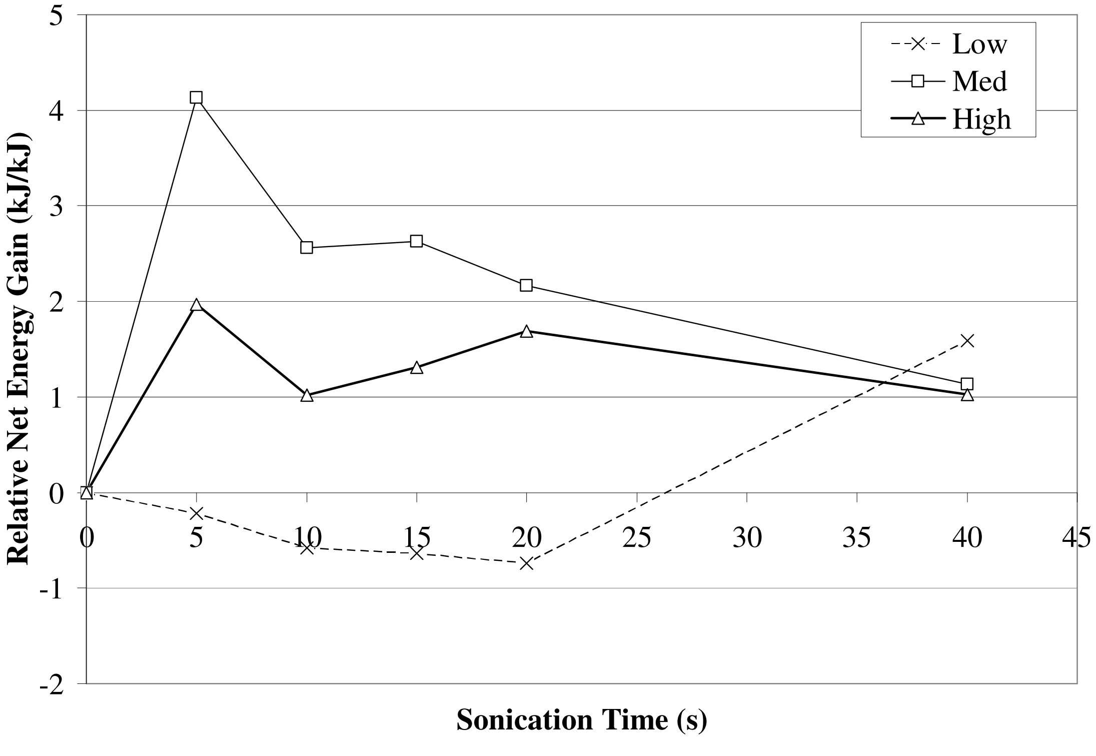


Figure 4.4 Ultrasonic relative net energy gain of sugary corn for varying ultrasonic power and varying sonication time

As shown in Figure 4.4, the ratio of the stored energy released (energy out) to the dissipated energy (energy in) exceeded more than 1.0 for the majority of the experimental design space, except at the low power setting and shorter sonication periods (≤20 s). It is believed that this could be due to under-treatment and inadequate gelatinization as well as lack of particle size reduction. For longer sonication times (>25 s), gelatinization increased to where the process became relatively efficient (>0%). At higher power settings, maximum energy gain occurred with a treatment time of approximately 5 s. The additional energy most likely did not reduce the particle size of starch granules further as described by Khanal et al. [17] nor promote additional gelatinization. Higher power settings had lower energy gain compared to medium power. This was attributed to denaturation/degradation of enzyme due to over-treatment.

Table 4.3 Ratio of treatment energy introduced versus the sugar released due to ultrasonication

	KiloJoule energy input / Grams sugar released		
Sonication Time (s)	**Low Power**	**Medium Power**	**High Power**
5	20.1	3.1	5.3
10	37.3	4.4	7.8
15	43.6	4.3	6.8
20	60	5.0	5.8
40	6.1	7.4	7.8

The results for energy input to sugar released ratio is shown in Table 4.3. The highest energy to sugar ratio was achieved by samples sonicated at low power for 20 s while the lowest was obtained by samples sonicated at medium power for 5 s. As the sonication time is prolonged, the energy required to release one gram of sugar has also increased. This trend is observed in low and medium power. At high power, the energy requirement varied as sonication time increases, but the difference was not as high as observed in low power. At low power, it is observed that a large amount of energy was required to release one gram of sugar. This could be associated to the negative net energy gain observed in Figure 4.7.

Polarized light microscopy and scanning electron microscopy (SEM)

During gelatinization, a critical step in converting the starch into sugar, intra-molecular bonds of starch are broken in the presence of water and heat. Because these experiments were conducted without temperature control, the final temperature in the reaction chamber increased in direct proportion to the power settings and the treatment time. Thus, it is believed that the ultrasonic energy partially gelatinized the starch allowing more effective enzymatic reactions. In order to confirm this hypothesis, a polarized optical microscopy examination was conducted to characterize sonicated and unsonicated (control) samples.

Figure 4.5 shows the polarized microscope images of sugary starch with sonication (B and D) and without sonication (A and C). Images 5C and 5D were viewed under a polarized microscope at 40x magnification. Images 5A and 5B were zoomed in 22.5x from Images 5C and 5D to focus only on a single Maltese cross pattern. The figure was identified as starch granule formation based on literature reports [21,22]. The control samples demonstrated a clear Maltese cross pattern that is normal for ungelatinized starch granules [23,21,22]. These patterns relate to semi-crystalline structures of starch and are often called spherulites. Spherulites and birefringence were more pronounced in the control groups (Figure 4.5A and 4.5C) compared to the sonicated groups (Figure 4.5B and 4.5D). The amount of birefringence correlates to the degree of crystallinity [21]. At the higher magnification (Images 5A and 5B), sonicated starch had no birefringence in the center of the spherulite [24] indicating that the sample had a lower degree of crystallinity and was partially gelatinized.

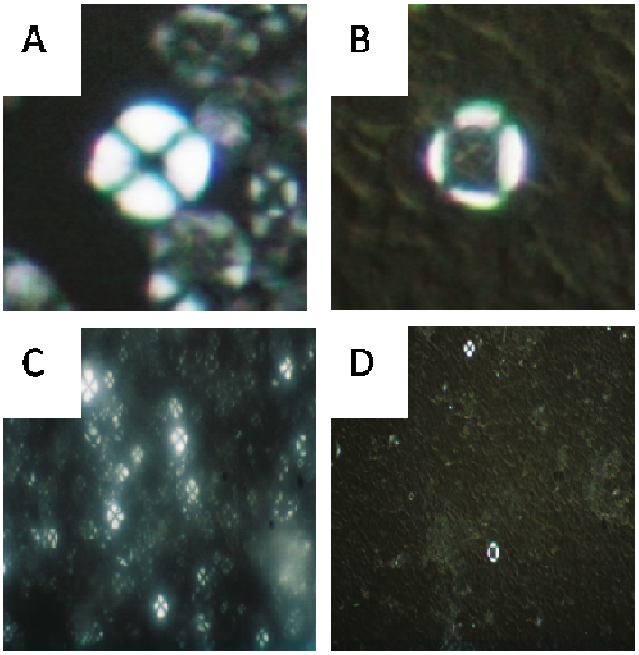


Figure 4.5 Polarized microscope images of control (A & C) and sonicated (B & D) samples. *Image 5C and 5D were viewed under a polarized microscope at 40x magnification while 5A & 5B were zoomed in 22.5x from image 5C & 5D. This is done to emphasize the maltese cross pattern in the starch granule.*

SEM images of corn slurries with and without ultrasonic treatment are shown in Figure 4.6. The images in the left column were magnified 300x, whereas the images on the right were magnified 1000x. In the control samples (Images 6A and 6B), the starch granules were intact. In contrast, as seen in a 20-s ultrasonic treated sample (Figure 4.6C), the starch granules were partially ruptured and at the higher magnification (Figure 4.6D) the granules were coated with what is believed to be gelatinized starch. Additionally, as the corn slurry was treated for 40s (Figure 4.6E and 4.6F), the starch granules were indistinguishable similar to what was found for commodity corn [17]. Furthermore, the samples appeared to be fully gelatinized.

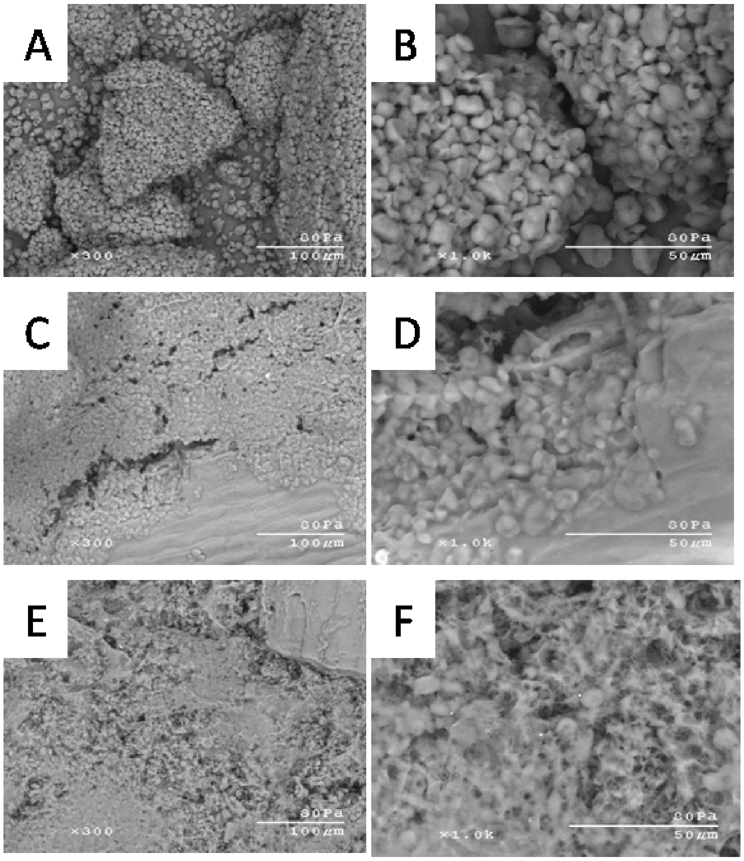
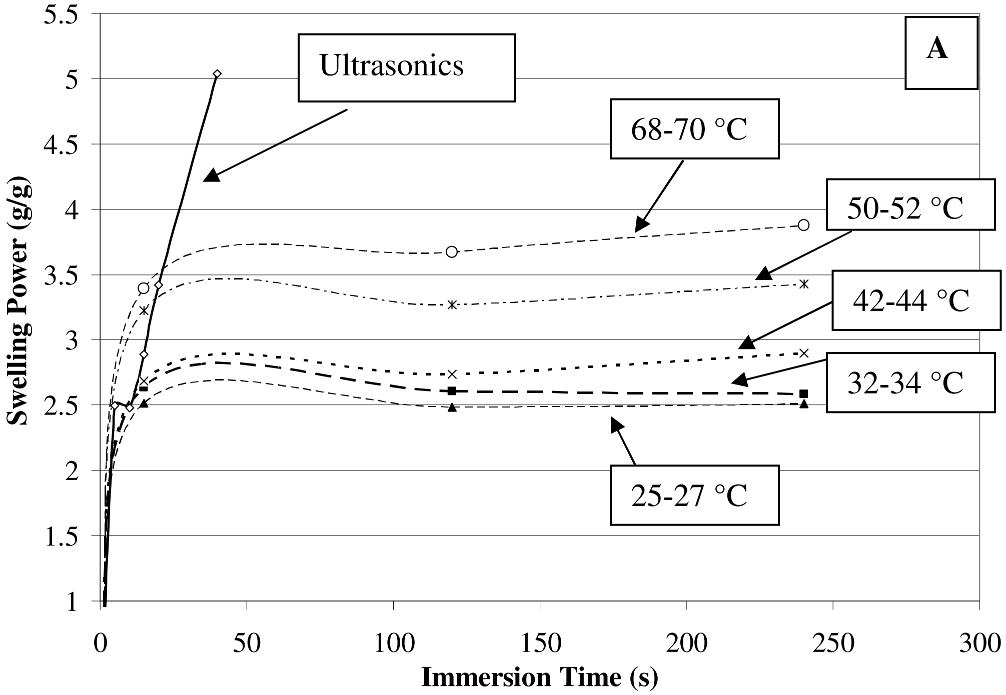


Figure 4.6 SEM images of control (A & B) and sonicated samples [for 20s (C & D) and 40s (E & F)]. *The images on the left column were magnified 300x while images on the right were magnified 1000x.*



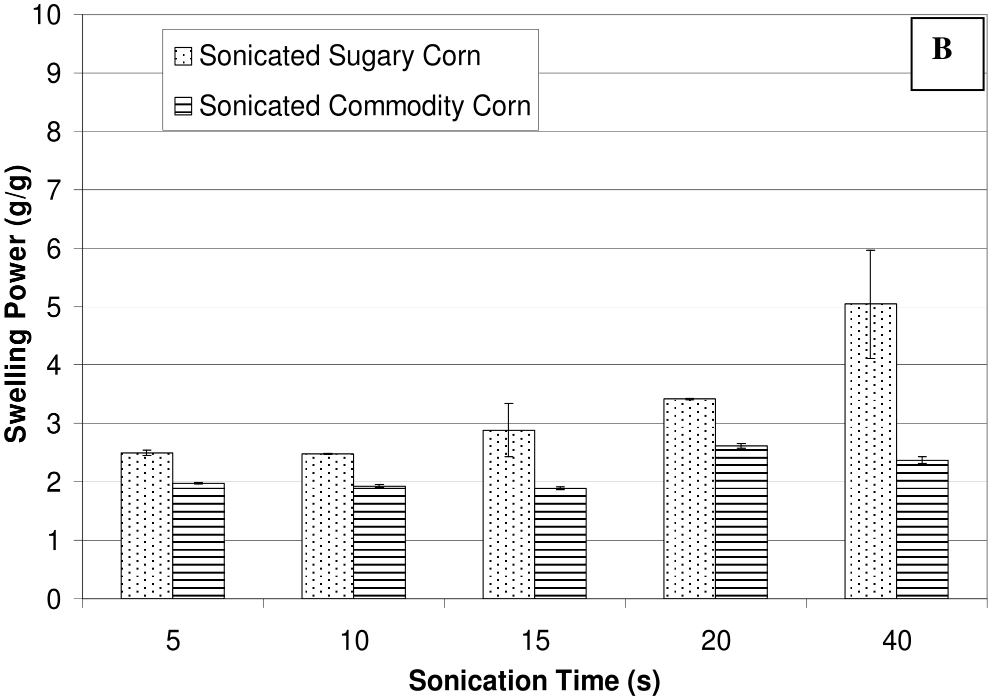


Figure 4.7 Swelling power comparison (A) between conventional heating and ultrasonics (B) between sugary and commodity corn

Swelling power of commodity corn and sugary corn

Based on earlier results, it was found that starch swelling and gelatinization occurs during ultrasonication. In order to confirm that ultrasonic energy introduced mechanical mechanisms that affect gelatinization, swelling power test was conducted and compared with conventional heating. The varied temperature of the conventional heat swelling test was based on the final temperature of the sample after ultrasonication (Table 4.2). The swelling power between conventional heating and ultrasonics is shown in Figure 4.7A. It is seen that the swelling power in the ultrasonicated sample initiated as quickly as 5 s and increased rapidly. These results are similar to that of Isono et al. [24] in which the rate of degradation of sonicated waxy rice starch was accelerated at or above the gelatinization temperature. For example, at the shortest ultrasonic treatment time, 5 s, the temperature was 25-27°C and at the end of the longest treatment time, 40 s, the temperature was 68-70°C. It is seen that in all cases, the swelling power is enhanced by the ultrasonic energy. In more detail, the rate of swelling (gelatinization) is higher with ultrasonic treatment compared to heat only. In addition, the final swelling power is substantially higher (~6 g/g) for the ultrasonic treatment compared to samples that were heated only (2.8 g/g). This increase is believed to be related to a finding of Huang et al. [25] that ultrasonics affected the starch amorphous regions and enhanced water absorption. In addition, Seguchi et al. [26] reported that ultrasonics gradually broke the starch agglomerate and reduced starch molecular sizes. This promotes the solubilization of the amylase in the suspension into a three-dimensional gel network [27].

Table 4.4 A comparison of water solubility index (WSI) between ultrasonicated sugary-2 corn and commodity corn; and between ultrasonication of sugary-2 corn and conventional heating treatment of sugary-2corn.

	Immersion Time (s)	**Water Solubility Index (%)**				
		Sugary Corn		**Commodity Corn**		
Ultrasonics	5	9.09		5.62		
	10	7.58		6.19		
	15	7.77		6.30		
	20	7.43		7.00		
	40	22.33		51.27		
Temperature (°C)		**25-27**	**32-35**	**42-45**	**52-55**	**68-72**
Conventional Heating (Sugary Corn)	15	4.55	4.53	2.86	4.05	3.23
	120	5.10	4.47	3.31	4.41	4.93
	240	5.27	4.59	3.69	4.72	6.09

Figure 4.7B shows the swelling power comparison between commodity corn and sugary corn. Commodity corn and sugary-2 corn were treated with ultrasonics at high power and varying sonication time. The swelling power of sugary corn-2 was observed to be higher than commodity corn in all ultrasonic treatment conditions. The swelling power for sugary corn and commodity corn ranged from 2.5-5.0 g/g and 1.9-2.6 g/g, respectively. Several studies reported that swelling power is lower for sugary-2 corn starches than normal corn starches [18,28]. On the contrary, this study found that sugary-2 corn has better swelling power than the commodity corn, which is comparable to the results found by Fuwa, et al. [29]. This could be explained by the fact that this study used ground corn instead of corn starch. That is to say, starch in this study is embedded in the corn kernel matrix. Thus, it may be more difficult to gelatinize than free granular starch. However with ultrasonic treatment the release of the starch was enhanced. Water solubility index (WSI) results for commodity and sugary corn are shown in Table 4.4.

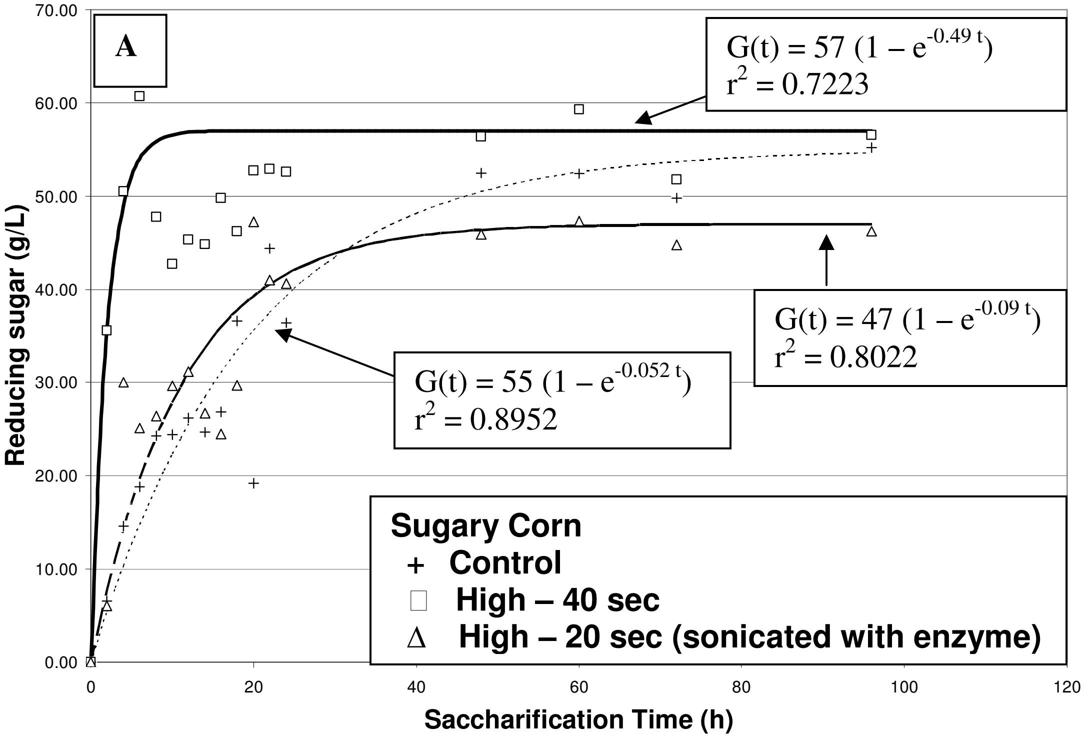
The WSI of commodity corn is 5-38% less than the WSI of sugary-2 corn. It is interesting to note the WSI of commodity corn at 40 s sonication time was 2x higher than the sugary-2 corn. However, conventional heating had considerably lower WSI than ultrasonics in treating sugary-2 corn.

Comparison of saccharification yield between commodity and sugary corn

To further characterize the effects of ultrasonics on the sugary corn slurry, experiments were conducted where the saccharification time was varied from 0 to 96 h. The samples were collected at 2-hour intervals during the first 24 hours and at 24-hour intervals after that. The samples were treated at the high-power setting for 20 and 40 s, the two most promising conditions. At the shorter treatment time (20 s), the Stargen™ enzymes were added to the corn slurry before ultrasonic treatment. Figure 4.8 shows sugar yield as a function of saccharification time for control and treated samples at the high-power setting for 40 s and high power for 20 s with enzymes, respectively. The data was then fitted with standard reaction rate kinetics using an Arrhenius model (Eq. 4.4):

$$G(t) = G_{\infty}\left(1 - e^{-kt}\right) \qquad \text{[Eq. 4.4]}$$

where *G(t)* is the glucose concentration (g/L) as a function of time (t), G_{∞} is the glucose concentration at time infinity, and *k* is reaction rate coefficient (h^{-1}).



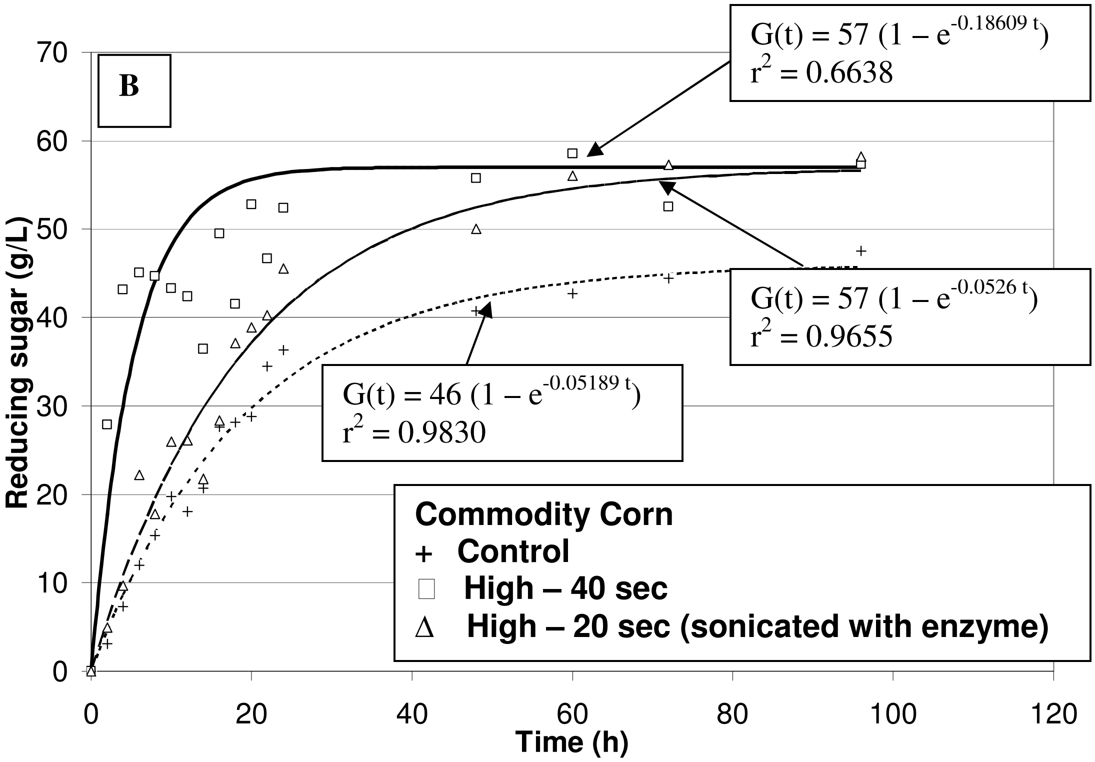


Figure 4.8 Sugar release at 96-h saccharification period (A) Sugary Corn (B) Commodity Corn

Figure 4.8A shows the sugar production of sugary-2 corn at varying saccharification time. The highest sugar production (60.7 g/L) of sugary-2 corn was achieved in samples sonicated at high power for 40 s, which is about 10% more compared to the untreated samples (55.2 g/L). Comparing this result with percent starch conversion data in Figure 4.2, it could be deduced that the first 3 hours of saccharification made a significant difference in the sugar production. As evident in Figure 4.8A, the sugar production in the sonicated sample (40 sec without enzyme) has almost completed saccharification in less than 20 hours while the unsonicated samples still continued saccharification. This could be due to the additional release of sugar in ultrasound pretreated samples. Some starch in the untreated samples could have been protected by surface proteins and lipids from enzyme attack, which was not present in the treated samples. The reaction rates of the sonicated samples (high power for 40 and 20 s sonication times) were 0.49 and 0.09, respectively, which are 2- to 10-fold higher than the reaction rates for the respective control group. Comparing the two ultrasonically treated samples, the reaction rate in the sample sonicated with enzyme present during treatment was five times lower than in the other sonicated sample. It is believed that this significant decrease in the sonicated samples with enzymes could be due to protein (enzyme) denaturation rendering them inactive during saccharification. Enzyme inactivation during ultrasound treatment was also observed in other studies [17,30].

Similar observations were made in Figure 4.8B for ultrasonically treated commodity corn. The highest sugar production was achieved by the sample sonicated for 40 s at 58.2 g/L, 22% lesser than the highest obtained by the control group at 47.5 g/L. It was observed that the sample sonicated at 40 s reached the highest production in 24 hours. Comparing the reaction rate coefficient, the ultrasonically treated sugary-2 corn was twice as fast as the ultrasonically treated commodity corn. It is evident that sugary-2 corn is easier to disintegrate than commodity corn. It is believed that this could be due to the arrangement of starch in the corn. Sugary-2 corn starches might have been easily released from the clusters of the endosperm than commodity corn starches. The reaction rate result is also in agreement with the swelling power test. However, the reaction rate values of the control groups for both type of corn were found to be comparable. Thus, without pretreatment, both sugary-2 and commodity corn could not be easily saccharified.

Conclusions

This study evaluated the effects of high-power ultrasonics in converting sugary-2 maize to fermentable sugars for ethanol production. The ultimate sugar release over a 96-hour period with an optimal level of ultrasonication has exceeded by 10% with respect to controls. The ultrasonic energy efficiency exceeded 100%, which indicated that more energy was produced in the form of chemical energy contained in sugar than the amount of ultrasonic energy introduced. SEM and polarized light microscope images confirmed that the ultrasonic treatments gelatinized the starch. When the saccharification time was extended to 96 hours, the reaction rates of the treated samples were 2-10 times higher than those of the control groups. It was also observed that under excessive treatment conditions, e.g. an exposure of 40 s at the highest power setting, the ultrasonication had adverse effect on the enzymes. This was not the case at the lower exposures and power settings: not only was the reaction rate higher but the final yield of sugar was also higher. It is concluded that optimized ultrasonic treatment enhanced the production of fermentable sugar from starch. Similar experiment was also conducted using commodity corn. It was found that treated sugary-2 corn saccharified faster than treated commodity corn. Therefore, it could be concluded that sugary-2 corn could be more easily disrupted compared to commodity corn. This study has also investigated the effect on swelling of ultrasonics in comparison with conventional heating. It has found that samples started to swell when subjected to ultrasonics even for as short as 5 seconds. The conventional heating initiated a higher swelling power at 30 seconds but increased at a lower rate than ultrasonics. Thus, this study has concluded that ultrasonics has better potential of swelling and gelatinizing the corn slurry than conventional heating. In addition jet cooking and ultrasonication obtained similar theoretical starch conversion results after 3 hours saccharification. Thus, it is evident that ultrasonication could be considered a potential alternative to jet cooking. It is important to note that future studies and implantation of this technology must consider the rapid gelatinization of the starch that may limit solids content of the slurry.

Acknowledgements

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CHAPTER 5: CHARACTERIZATION OF PARTICLE SIZE AND SACCHARIFICATION IN BATCH AND CONTINUOUSLY ULTRASONICATED CORN SLURRY

A paper to be submitted to *Biotechnology Progress*

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Abstract

The effects of ultrasound on corn slurry in terms of saccharification yield and particle size distribution was studied. Two independent ultrasonic experiments were conducted at a frequency of 20 kHz; in batch and continuous flow systems. Ground corn slurry (33% m/v) was prepared and sonicated in batches at various amplitudes (192-320 $\mu m_{\text{peak-to-peak (p-p)}}$) for 5, 10, 15, 20 and 40 s using a standard catenoidal horn. The ground corn slurry (33% m/v) was pumped at various flow rates (2.7-7.4 gal/min or 10.2-28 L/min) in continuous flow experiments, and sonicated at constant amplitude ($12\mu m_{p\text{-}p}$). In these experiments the corn slurry was pumped through a reactor equipped with a Branson Ultrasonics "donut" horn. After ultrasonic treatment, STARGEN^{TM} 001 was added to the

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samples then saccharified for 3 hours. The treated samples were found to yield 2-3 times more reducing sugar compared to the control (untreated). Sonication at medium amplitude (256 μm_{p-p}) for 40s was found to be the optimal in batch mode. Although the experiments conducted in continuous mode released less reducing sugar than the batch mode, the continuous mode was more energy efficient. The particle size of the sonicated corn slurry (both batch and continuous modes) was reduced relative to the control (no sonication). The reduction of particle size was proportional to the energy introduced by the ultrasonic treatment. Scanning electron microscope images showed substantial changes in the morphology of the treated corn slurry particles. Overall, the study suggest that both batch and continuous flow ultrasonic modes enhanced saccharification yield, reduces particle size of corn slurry and could a potential pretreatment for corn slurry.

Keywords

Corn Slurry, Reducing Sugar, Saccharification, Ultrasonics, Particle Size, Morphology, Enzyme Hydrolysis, Ethanol

Introduction

Ultrasonic technologies have been used in diverse applications for decades. Ultrasound is the range of sound waves at a frequency above the normal upper human hearing limit (> 18-20 kHz). As ultrasound waves propagate in a liquid medium, cavitation occurs [1,2]. “Cavitation is the production of microbubbles in liquid when a large negative pressure is applied to it” [3]. Because of surface tension and the presence of other bubbles, foreign bodies, and gradients in the pressure waves, each bubble becomes unstable beyond a critical size and eventually collapses violently [3]. As the bubbles collapse, localized temperatures reaching up to 5000 K and pressures up to 180 MPa can be achieved [4]. Ultrasound waves in liquid media also produce acoustic streaming, which is used to facilitate the uniform distribution of ultrasound energy in the media, and convection of the liquid, and dissipation of any heat produced [5,2].

The use of ultrasonics in starch hydrolysis has been reported as early as 1933 by Szent-Gyorgyi [6]. Ono [7] conducted a similar study using ultrasonics in disintegrating potato and wheat paste. In starch solutions, depolymerization of large molecules can occur as a result of ultrasonic treatment [8,9]. More specifically, ultrasonic energy is capable of depolymerizing macropolymers such as starches. In a study by Isono et al. [10], ultrasonic effects on waxy rice starch were determined by average molecular weight and molecular weight distribution using gel permeation chromatography. They found reduction in the average molecular weight of rice starch as a result of ultrasonication [10]. Similar results were obtained by Seguchi and company [9] as the initial starch average molecular weight decreased from $25x10^4$ to $1x10^4$ after sonication. In these cases, the cavitation breaks the primary back-bone bonds resulting in smaller molecules. When ultrasound was used to treat rice grains, particle size reduction resulted in shorter cooking times and faster gelatinization [11]. In addition, Zhang et al. [12] used ultrasound to enhance fractionation of yellow dent corn into its components (starch, germ, fiber, gluten). The study showed higher starch separation and lower protein content in the resulting starch fraction.

Wood et al. [13] found that ultrasonics enhanced the production of ethanol and reduced the use of enzymes during the simultaneous saccharification and fermentation of mixed office wastepaper. Khanal et al. [14] also reported promising results using ultrasonics treating corn slurry for ethanol production. The study used a bench-scale batch ultrasonics system to pretreat commodity corn to enhance sugar release during saccharification. A similar study was also conducted using sugary-2 corn as the substrate [15]. They found that ultrasound treatment gelatinized the corn starch and helped enzyme hydrolysis.

Even though batch ultrasonics proved to be an effective pretreatment of corn slurry for ethanol production, it is not a suitable system for industrial scale. This study was intended to evaluate the potential use of a continuous flow ultrasonic set-up using a “donut” shaped horn for possible large-scale systems. These radial horns have been used in large-scale pretreatment of waste activated sludge (biosolids) for enhanced anaerobic digestion [16]. Using the continuous flow set-up, the fluid is pumped through the center of the radial horn where it is exposed to intense cavitation. Because the fluid is moving through the center of the horn, it enhances the interaction of the slurry with the collapsing bubbles. This system uses transducers designed for continuous duty cycles, which makes it suitable for large-scale systems. The retention time can be controlled by the flow rate of the fluid.

This research focused on the pretreatment of corn slurry for enhanced saccharification using batch and continuous-flow ultrasonic systems. The objective of the study was to compare the two ultrasonic modes in terms of sugar release, relative energy gain and particle size reduction. Because maximum deliverable power is inversely proportional to frequency, we selected to study relatively low frequencies (20 kHz) in both ultrasonic systems.

Materials and Methods

Ultrasonic Batch Experiments

Corn slurry samples were prepared using 33% (m/v) dry-ground corn in water (Lincolnway Energy, Nevada, IA). Twenty-five (25) ml corn slurry was added to 10 ml 0.1M acetate buffer, pH 4.5. The enzyme used was STARGEN^{TM} 001 (456 granular starch hydrolyzing units in GSHU/g) from Genencor International (Palo Alto, CA), which contained *Aspergillus kawachi* α-amylase expressed in *Trichoderma reesei* and glucoamylase from *Aspergillus niger* that hydrolyzes starch dextrins into glucose. The composition of the corn was obtained using a Near-Infrared (NIR) Infratec^{TM} 1241 Grain Analyzer (FOSS Tecator, Eden Prairie, MN).

Corn slurry samples were sonicated using a Branson 2000 Series bench-scale ultrasonic unit (Branson Ultrasonics, Danbury, CT) for 20 and 40 s. The control samples were not subjected to sonication. The system operates at a maximum power output of 2.2 kW and a frequency of 20 kHz. The ultrasonic treatments were carried out in 50 ml polypropylene centrifuge tubes using three different amplitudes (power): low (192 μm_{pp}), medium (256 μm_{pp}), and high (320 μm_{pp}). The corresponding power output at the three amplitudes are 140-153 W (low), 214-228 W (medium), and 199-298 W (high). The horn was a standard 20 kHz half-wavelength catenoidal titanium with a flat 13 mm diameter face (gain = 1:8). STARGEN^{TM} 001 enzymes (30 μl) were added after sonication. The same amount of enzyme was added to the control samples. All the samples were then incubated for a shortened saccharification period of 3 hours in a rotary shaker at 150 rpm and 32°C.

Ultrasonic Continuous Flow Experiments

Continuous flow experiments were conducted using Branson 2000 Series bench-scale ultrasonic unit (Branson Ultrasonics, Danbury, CT) operating at 3 kW and 20 kHz. Corn slurry with 33% (m/v) ground corn concentration was pumped from a continuously stirred feed tank to an ultrasonics reactor where the Branson Ultrasonics "donut" horn was installed (Figure 5.2). Volumetric flow rates were varied from 10.2 (2.7 gal/min) to 28 L/min (7.4 gal/min). Constant ultrasonic amplitude of 12 $\mu m_{p\text{-}p}$ was used. Thirty microliter (30µl) of STARGEN™ 001 enzyme was added after ultrasonic treatment. For the experiments that involved saccharification, 25 ml treated corn slurry was added to 10 ml 0.1M acetate buffer pH 4.5. The control sample was not treated with ultrasonics. All the samples were incubated (liquefied and saccharified) for 24 hours in a rotary shaker at 150 rpm and 32°C. Figure 5.1 & Figure 5.2 shows the schematic of the experimental setup and the "donut" shaped horn. In order to assure proper mixing and reduce fouling of the pump, the corn slurry was continuously mixed in the feed tank as well as recirculated using the pump. The corn slurry flow rates are varied by adjusting the opening of the flow control and recirculation valves.

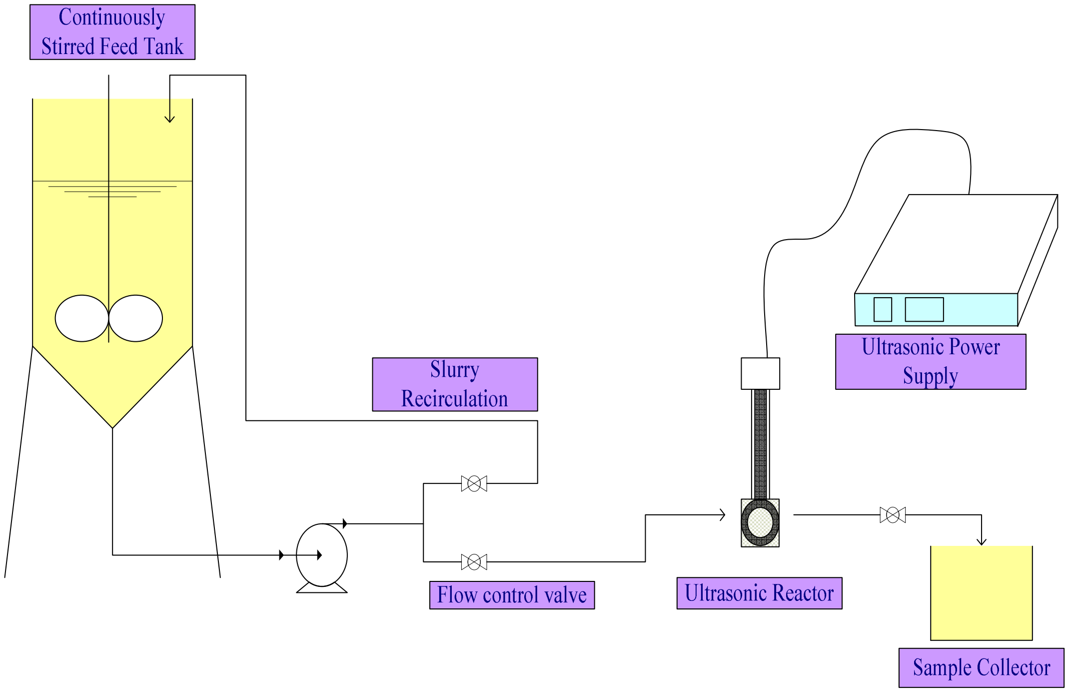


Figure 5.1 Ultrasonic Continuous Flow Experimental Set-up

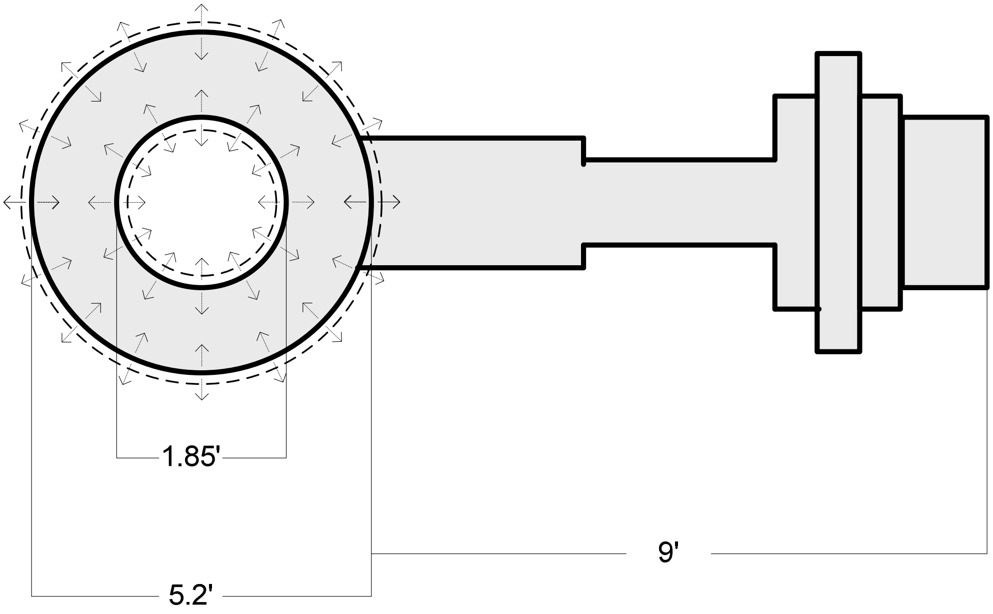


Figure 5.2 Branson Ultrasonics "donut" shaped horn

Figure 5.2 shows the donut shaped structure of the ultrasonic horn with its actual dimension. As seen in the figure, the horn vibrates in radial direction. In this study, the horn was placed in vertical position inside a reactor chamber where fluid passed through the center of the horn and some flows around it.

Analytical Procedures

After incubation, 2 ml 4M HCl Tris buffer (pH 7.0) was added to the samples to terminate enzyme activity. Samples were centrifuged at 2,500 rpm for 15 minutes. Supernatant was then analyzed for reducing sugar concentration (glucose as standard) using a modified dinitrosalicylic acid (DNS) method [17,14]. The particle size distribution of the corn slurry before and after ultrasonic treatment at different power levels was determined using a Malvern particle size analyzer (Mastersizer 2000, Malvern Inc., Worcestershire, United Kingdom). In addition, scanning electron microscopy (Hitachi S-2460N VP-SEM, Hitachi, Ibaraki, Japan) was used to examine the corn slurry samples.

Ultrasonic Relative Net Energy Gain

The energy gain was established by comparing the total energy delivered during sonication (Energy in, E_{in}) to the chemical energy of the additional sugar produced relative to the control group (Energy out, E_{out}). By assuming D-glucose as the standard monosaccharide used for reducing sugar analysis, and by further assuming the energy density of glucose (15,740 kJ/kg), the total energy dissipated into the sample (E_{in}) in batch and continuous flow ultrasonic systems can be described by Eqs.5.1 and 5.2. The overall ultrasonic relative net energy gain is shown in Eq 5.3.

$$E_{in} = \int_{t_0}^{t_f} P dt \sim E_{in} = P_{avg} t \qquad \text{[Eq. 5.1]}$$

$$E_{in} = \frac{P_{avg}}{Vfr} \qquad \text{[Eq. 5.2]}$$

$$Gain = \frac{E_{out} - E_{in}}{E_{in}} \qquad \text{[Eq. 5.3]}$$

Where: t_0 and t_f are the initial and final sonication exposure (seconds)

Vfr is volumetric flow rate (liters/seconds)

P_{avg} is average power (Watts)

Statistical Analysis

All experiments were performed in duplicates and analytical analysis in triplicates. Analysis of variance using MyStat 12 (student version of Systat) were used to compare the mean reducing sugar concentrations among various treatments with 95% significance level ($p<0.05$).

Results and Discussion

Saccharification Yield

The reducing sugar yield of treated and untreated corn slurry from the batch mode at various treatment times is shown in Figure 5.3. The sonication time of zero (0) corresponds to the control group (untreated sample). It is seen that ultrasonics enhanced saccharification of corn slurry as evident of the higher reducing sugar yields. In more detail, the treated sample yielded 0.4-3.3 times as much sugar compared to the untreated sample (control). It was also seen that the additional sugar release is proportional to the ultrasonic power level (amplitude) and the sonication time. It is interesting to note that medium and high power settings with a treatment of 40s differed only by 2%. Statistical analysis showed that there is no significant difference between the means. It is believed that this corresponds to a saturation of ultrasonic energy. In more detail, further increase in power setting or sonication time did not increase the sugar release. Based on these observations it was assumed that the medium power setting with a treatment time of 40 s corresponds with the highest saccharification yield over the conditions studied. This result is in agreement with the previous findings [14].

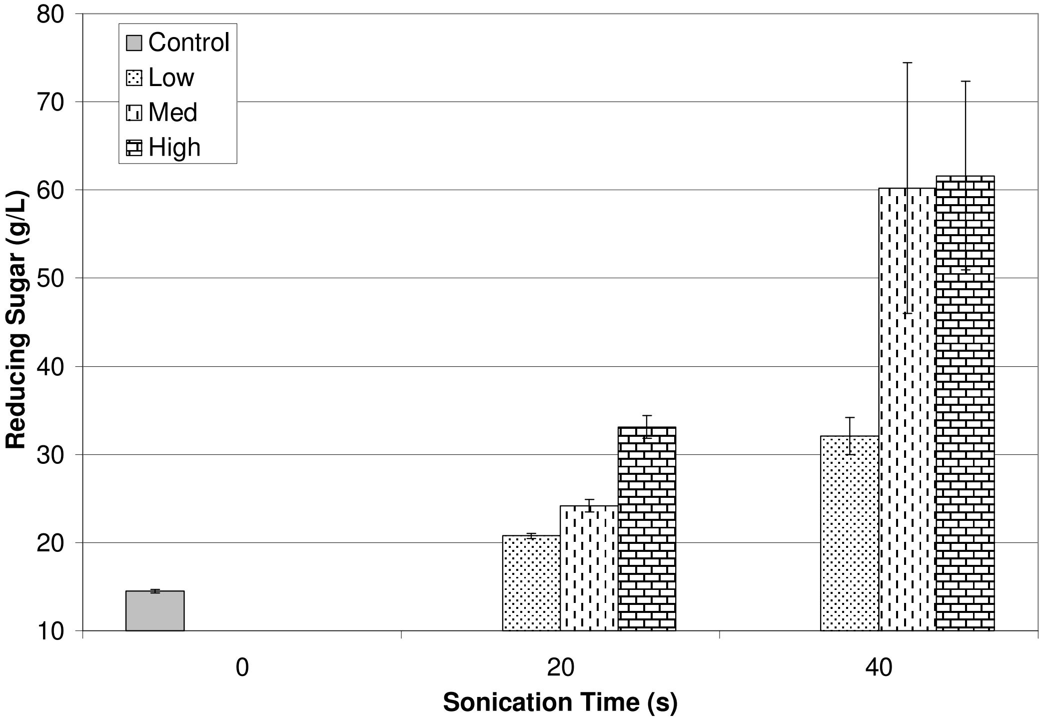


Figure 5.3 Reducing Sugar Yield of Treated Corn Slurry in Batch Ultrasonic System after a 3-h Saccharification Period

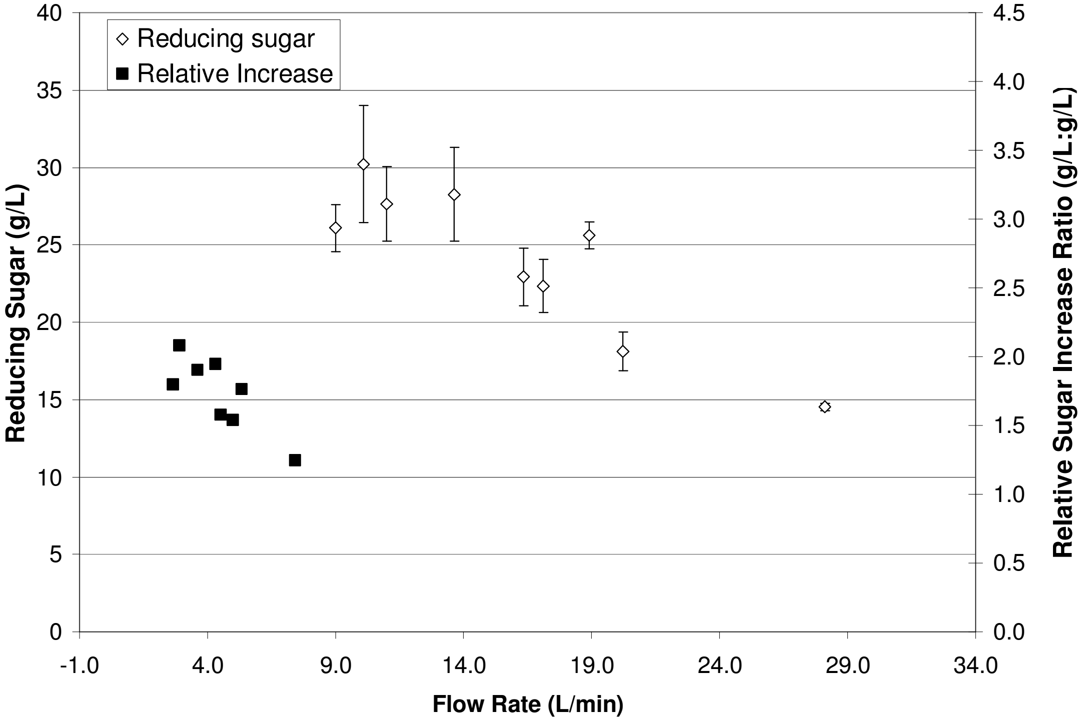


Figure 5.4 Reducing Sugar Yield and Relative Sugar Increase in Treated Corn Slurry in a Continuous Ultrasonic System after a 3h Saccharification Period

Figure 5.4 shows the yield of reducing sugar and relative sugar increase of treated corn slurry in a continuous flow ultrasonic system with a 3-h saccharification period. The volumetric flow rate was varied from 10.2 to 28 L/min (2.7-7.4 gal/min). In this study, a flow rate at 0 gal/min was assumed to correspond with the control sample (untreated). It is seen that the treated sample yielded between 24-100% more sugar compared to the untreated samples. The relative sugar increase (secondary y-axis) is the ratio of the sugar released by the treated to the untreated sample. The highest relative sugar increase ratio was 2.1 with a flow rate of 10.9 L/min (2.9 gal/min). This suggests that ultrasonics can enhance sugar yield by a factor of 2, compared to the untreated sample. The relative sugar increase ratio fluctuated around 1.5-2.1 over the majority of the flow rates that were studied. However, at a flow rate of 28 L/min (7.4 gal/min), the ratio dropped to 1.25. This could be due to lower retention time at higher flow rate, resulting in insufficient ultrasonic exposure.

Table 5.1 Comparative Tabulation of Batch and Continuous Flow Ultrasonic Systems in Terms of Energy Density and Starch Conversion

Batch Ultrasonics System				**Continuous Flow Ultrasonics System**		
Power Level	**Treatment Time (s)**	**Energy Density (kJ/l)**	**Starch Conversion**[i] **(%)**	**Flowrate (L/min)**	**Energy Density**[ii] **(kJ/l)**	**Starch Conversion**[i] **(%)**
Low	20	87.7	12.8	10.1	12.4	16.3
				11.0	11.4	18.8
	40	160	19.8	13.6	9.2	17.2
Med	20	122.3	14.9	16.3	7.7	17.6
				17.1	7.3	14.3
	40	260.6	37.1	18.9	6.6	13.9
High	20	170.3	20.4	20.2	6.2	16.0
	40	227.4	37.9	28.1	4.4	11.3

[i] Average starch conversion at 3h saccharification period

[ii] Assumption: Ultrasonics is 70% efficient

To compare the two modes (batch and continuous flow) in terms of saccharification, Table 5.1 tabulates their relative energy density and starch conversion. It is seen that the energy density for the continuous flow mode is nearly 20 times lower compared to the batch mode. This is due to the 27-fold difference in the amplitude of the two modes. The highest starch conversion yield of the batch and continuous flow modes were 37.9% at 227.4 kJ/L and 18.8% at 13.1 kJ/L, respectively. It is important to note that this is for a limited saccharification time (3 h). In addition, while batch mode yielded twice as much sugar, its energy dissipation was 17 times higher. The lowest starch conversion yield achieved by the continuous system was 11.3% using 5.1 kJ of energy per unit volume. Comparing this number with the batch system, it took the batch system 87.7 kJ/L to achieve a 12.8% starch conversion. This indicates that less energy density is required in a continuous flow system to achieve almost the same amount of starch conversion as in the batch system.

To further compare the two modes, three of the highest relative net energy gains as defined in previous work [14,15] are shown in Figure 5.5. All the treatment variations show a positive relative net energy gain. It is evident that the continuous flow mode is more efficient compared to the batch mode (24 compared to 16 (J/L)/(J/L). This is due to the very high power input and low working volume in the batch systems.

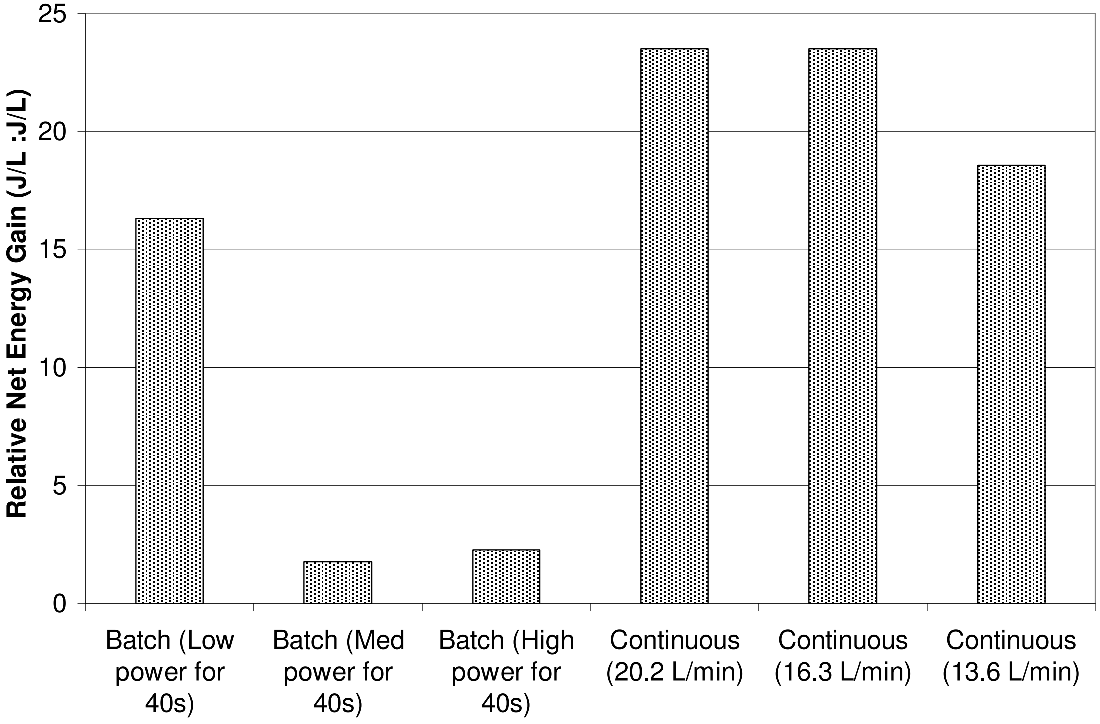


Figure 5.5 Three of the Highest Ultrasonic Relative Net Energy Gains in Batch and Continuous Flow Ultrasonic Systems

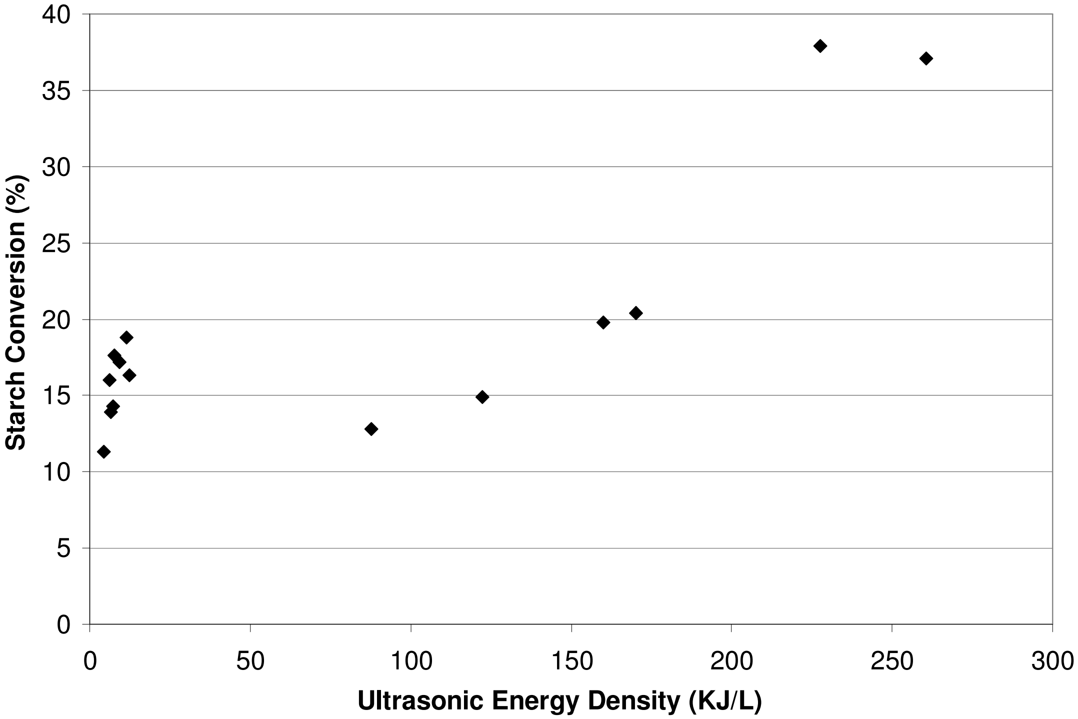


Figure 5.6 Starch Conversion and Ultrasonic Energy Density of Batch and Continuous Flow System

Figure 5.6 shows the starch conversion as a function of ultrasonic energy density for the batch and continuous flow systems. As seen in the figure, the continuous flow energy densities span a smaller range of energy densities 4-15 kJ/L while the batch system range from 70-260 kJ/L. As expected, the starch conversion increase was proportional to the rise in ultrasonic energy density. It is interesting to note that the continuous flow system has a higher dependence on starch conversion and energy density relative to the batch system. It is seen, the continuous system has 18.8% starch conversion with an energy density of 11.4% while batch system has 12.8% with an energy density of 87.7 kJ/L. This suggests that the continuous system is more efficient in converting starch compared to the batch systems.

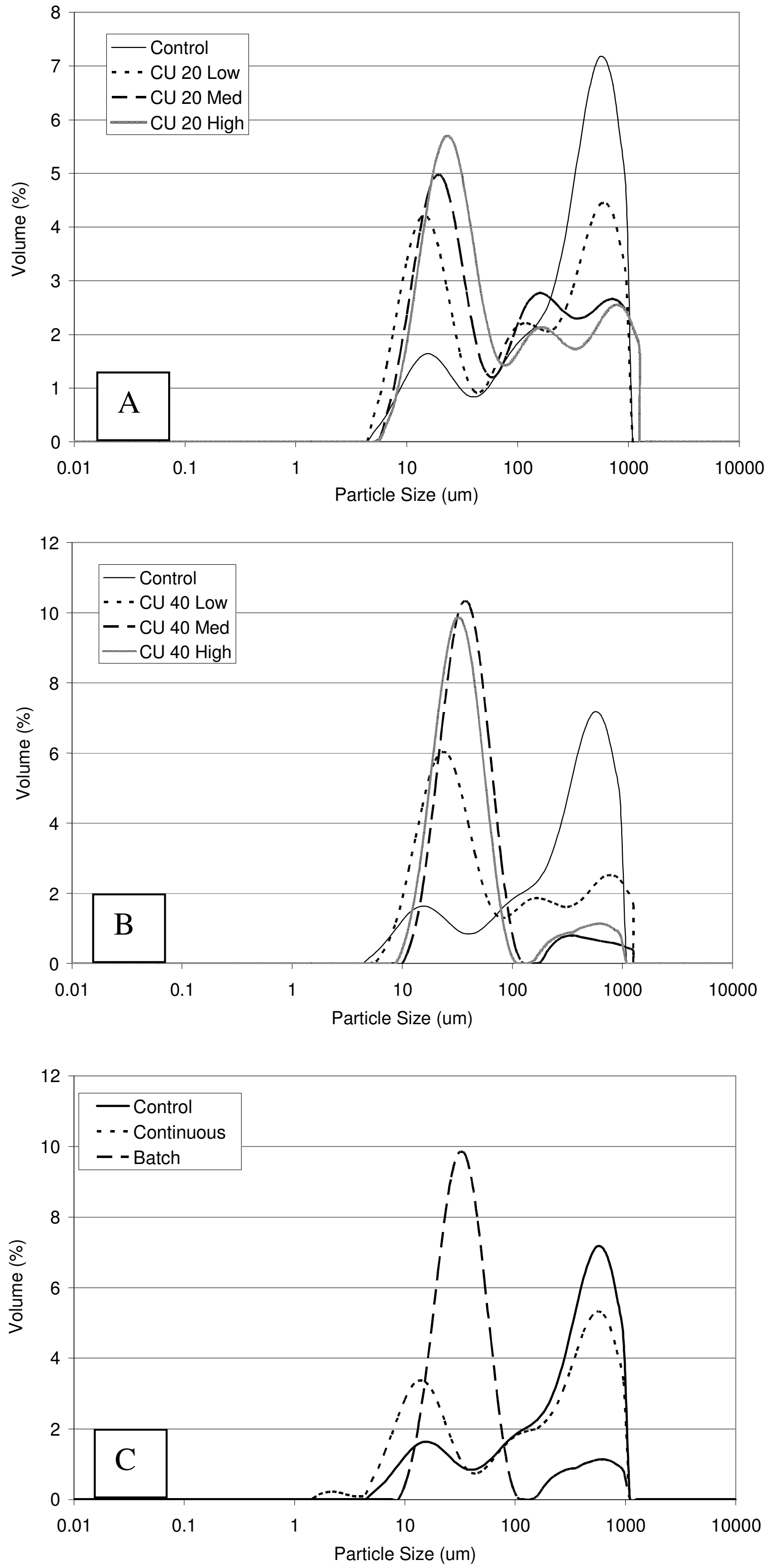


Figure 5.7 Particle Size Distribution in Batch and Continuous Flow Experiments: (A) Low Power (B) Medium Power (C) High Power

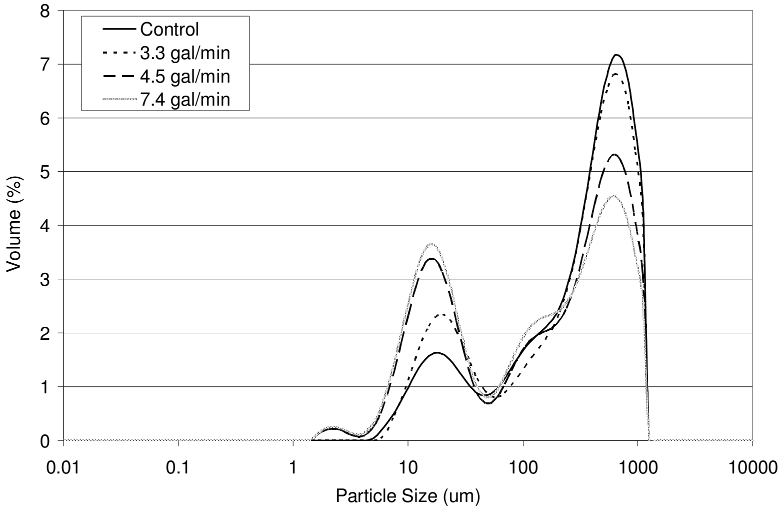


Figure 5.8 Particle Size Distribution of Continuous Flow Experiments at varying flowrate

Particle Size Characterization

Several studies have investigated the effects of ultrasonics on particle size [12,18]. In this study, the results of ultrasonic treatment in batch and continuous flow experiments were compared with non-sonicated samples (control). Because 1200 µm is the maximum particle size measured by the Malvern Mastersizer, the samples were screened through a 1000 µm screens prior to analysis. Figure 5.7A and B compare the control sample to the samples sonicated at low, medium, and high power, for 20 and 40 s, respectively. As seen, the particle size distribution has 2 inflection points in the control while in contrast there are 3 inflection points in the sample sonicated for 20s. Interestingly, it is seen that with the 40 s treatment there is a single modal distribution. Additionally, the inflection point of the particle size distribution curve is shifted from 500 µm to approximately 20 µm following sonication. It was also seen that the relative volume within the inflection point increased as ultrasonic power level increased, for both

20-s and 40-s sonication times. This data is in good agreement with Khanal et al. [14] where particle size was found to be generally inversely proportional to ultrasonic treatment energy. Figure 5.8shows the particle size distribution of the continuous flow experiment at varying flowrates. Three flowrates were taken and compared with the control (unsonicated). It was observed that particle size reduction is similar to the increasing flowrate of the continuous flow experiment. In Figure 5.7C, the particle size distribution in the batch mode is compared with particle size distribution for the continuous flow mode. The highest particle size reduction was found at a 7.4 gal/min (28 L/min) flowrate (Figure 5.8), thus it was used in this plot for comparison. It is important to note that these results are similar to the results for reducing sugar in the continuous flow samples. In more detail, only the particle distribution showed a different volume fraction.

It is believed that the inflection points at <100μm corresponded to cell morphologies of the corn. In order to gain insight into the composition and morphology of the various particle size distributions, SEM images were obtained. After sonication, the corn slurry samples were centrifuged at 2,500 rpm for 15 minutes. Assuming that due to lesser specific frictional drag, the larger particles would settle first during centrifugation and the finer particles last, the pellet (solid fraction after centrifugation) was divided into three parts. Because it was observed that the corn slurry sonicated for 20s at high power produced three easily distinguished and separable layers, it was used for these studies. Figure 5.9(A and B) show the SEM images from the top and middle layer while Figure 5.9 (C and D) show the bottom layer. After centrifugation, it is seen that the top layer has morphology similar to a gel. It is believed that this corresponds to the portion of the corn that was gelatinized by ultrasonication, as also seen in Figure 5.9A.

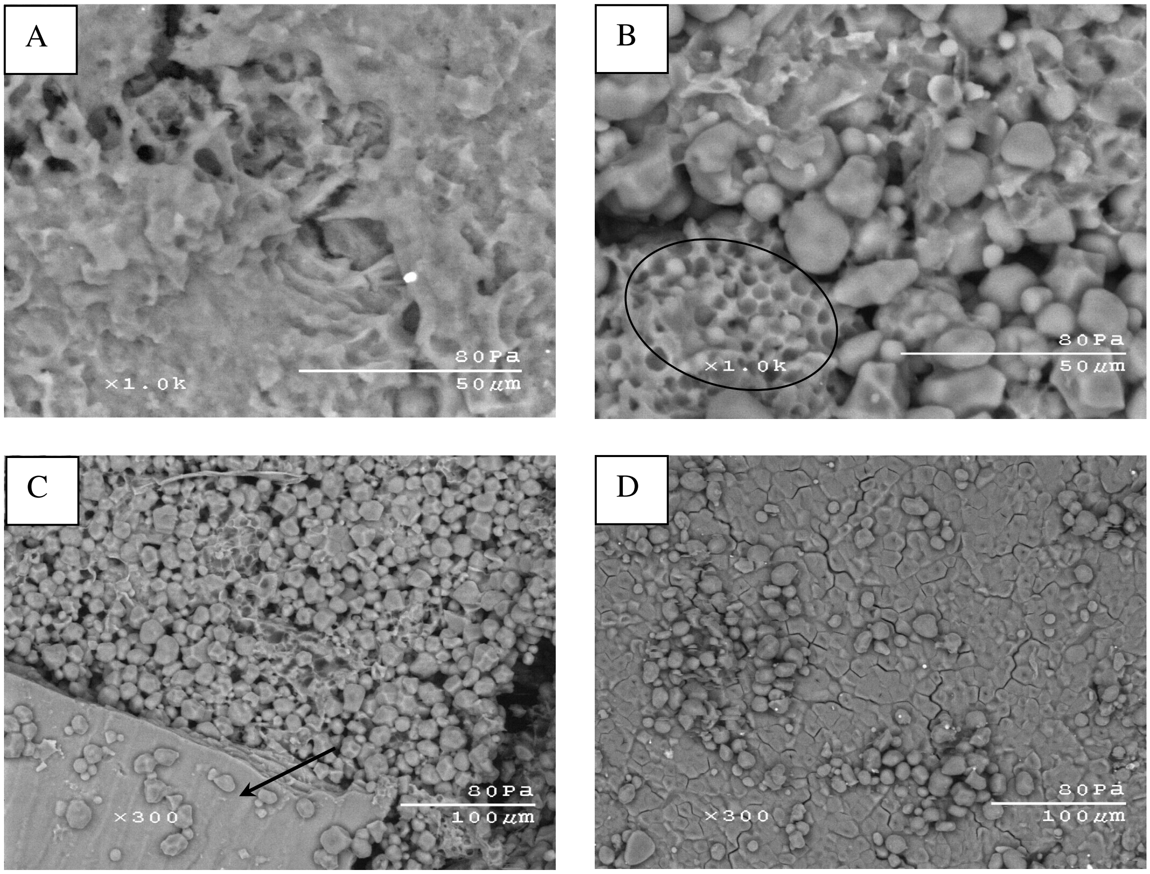
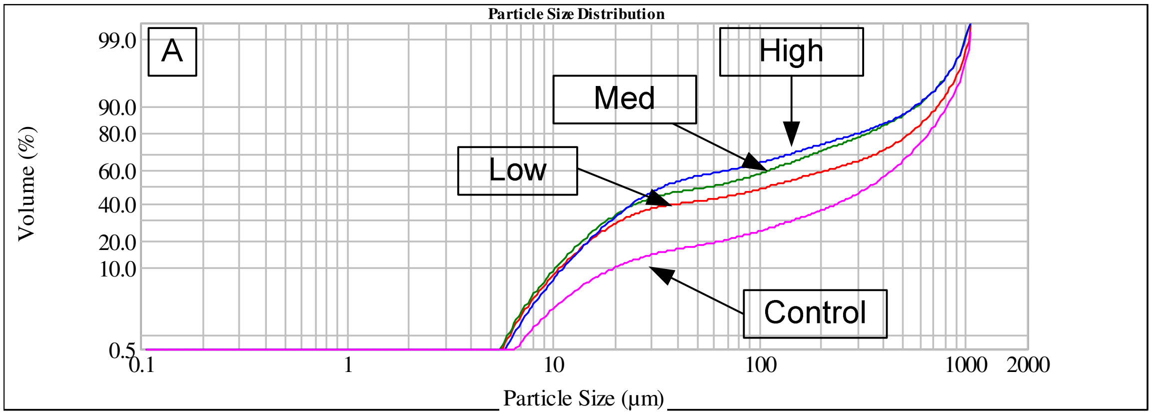


Figure 5.9 SEM Images of Sonicated Corn Slurry: (A) Top Layer (B), 2nd Layer, (C,D) Bottom Layer

Because corn kernels compose of three main parts; pericarp, endosperm, and germ, it is anticipated that the each part would present themselves differently after sonication. In more detail, the pericarp, sometimes referred to as the "hull," contains the cellulosic and hemicellulosic fraction of the kernel, which is relatively tough compared to the balance of the kernel. As seen in the SEM image, Figure 5.7A shows a gel-like structure which was assumed to be the gelatinized part of the starch [15]. In Figure 5.9C, a large hard portion of the pericarp (indicated by the arrow) is found among the clusters of starch granule. Upon extracting of the middle and bottom layers, similar particle morphology was seen. This suggests that the middle and bottom fraction contain the endosperm fraction. According to Hoseney [19], there are two types of corn endosperm, translucent and opaque. The translucent endosperms are tightly packed, with few voids [19]. Their starch granules are polygonal in shape and held together by a protein matrix [19], which is similar to the structure seen in Figure 5.9D. These proteins are typically referred to as zein bodies [19]. Opaque endosperms are spherical in shape, highly spaced within one another, and also covered with a protein matrix [19]. This is observed in Figure 5.9B, C, and D. Another particular formation found was a honeycomb-like structure, as seen in Figure 5.9B. It was noticed that the cells are hollow and thin walled. It is believed that these correspond to compacted cells. In more detail, Hoseney [19] explained that a possible reason for the differences in the shape of the corn endosperm is that in the natural drying process, “the protein loses water and shrinks”. “The adhesion between the protein and the starch is strong enough to pull the starch granules closer together” [19], resulting in the tightly packed polygonal shape. Based on these observations, the second and third fraction could correspond to the second (150 μm) and third peaks (800 μm). It is deduced that after further sonication, these starch clusters are further disintegrated, resulting in free individual starch granules with sizes of 10-20 μm.



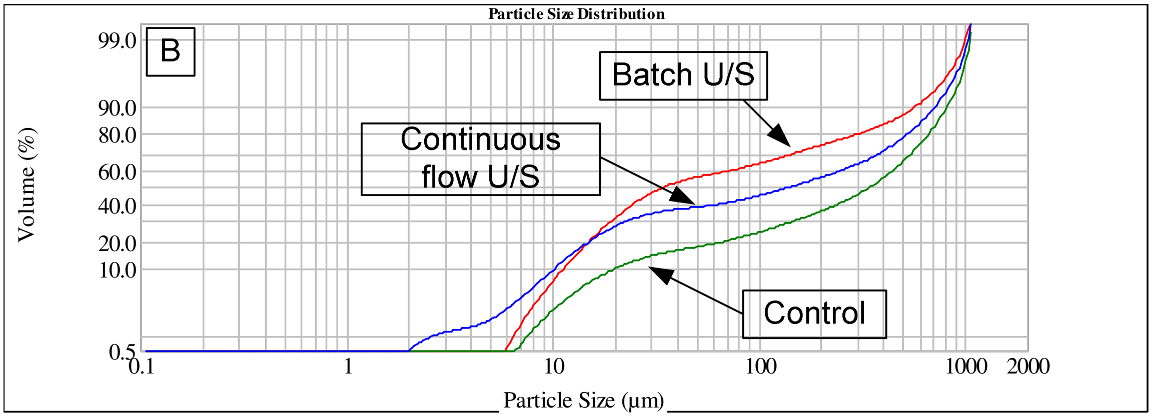


Figure 5.10 Particle Size Distribution of Corn Slurry in Log-Probability Plot: (A) Varying Power Level of Batch Ultrasonic System (B) Batch and Continuous Ultrasonic Systems

Typically, particle size distribution of particulates emissions in air are characterized using a log-probability distribution. When the particle sizes are plotted on a logarithmic scale against the frequency of occurrence, it generates a bell-shaped curve called the lognormal curve [20]. The particle size distributions of the corn slurry in terms of its probability percentage are shown in Figure 5.10. According to the EPA module on particle size distribution [20], a straight line indicates a log-normal data set. In this study, lognormal curves were obtained using Malvern Mastersizer 2000 software ver. 5.4

(Malvern Intruments, Ltd). Figure 5.10A is the probability plot of corn slurry at varying power level of batch system while Figure 5.10B is the comparison between batch and continuous flow systems. It could be seen that the lognormal line is directly proportional to the ultrasonic power level in batch systems (Figure 5.10A). It is evident that control has a lower lognormal trend line compared to the ultrasonically treated samples. This signifies the particle size reduction of corn slurry due to ultrasonication. Similar trend is observed in Figure 5.10B where both ultrasonication systems had higher trend line than the control group. With the higher energy input in batch systems, it was found to have more particle size reduction than continuous flow systems.

In Figure 5.10, it is evident that the particle size distribution is not lognormal. At 50% probability, the particle size of control (without sonication) was 361.8 μm while particle size of low, medium and high were 115.2 μm, 61.0 μm and 35.2 μm, respectively. Similarly, in Figure 5.10B the particle size of continuous flow sonication at 50% probability was 195.1 μm. The values indicate that at 50% probability, the particle size reduces as sonication treatment increases.

Also, the volume weighted means (VWM) was correlated to the energy density dissipated by the ultrasonic treatment in the batch system. Figure 5.11 shows the VWM as a function of energy density (energy per unit volume, kJ/L). It is seen that the VWM is inversely proportional to the dissipated energy. This correlation was expected because as the energy increased, more particles are disintegrated. When a model trend line was drawn, a good r^2 fit of 0.9147 and negative slope of 0.9863 was found.

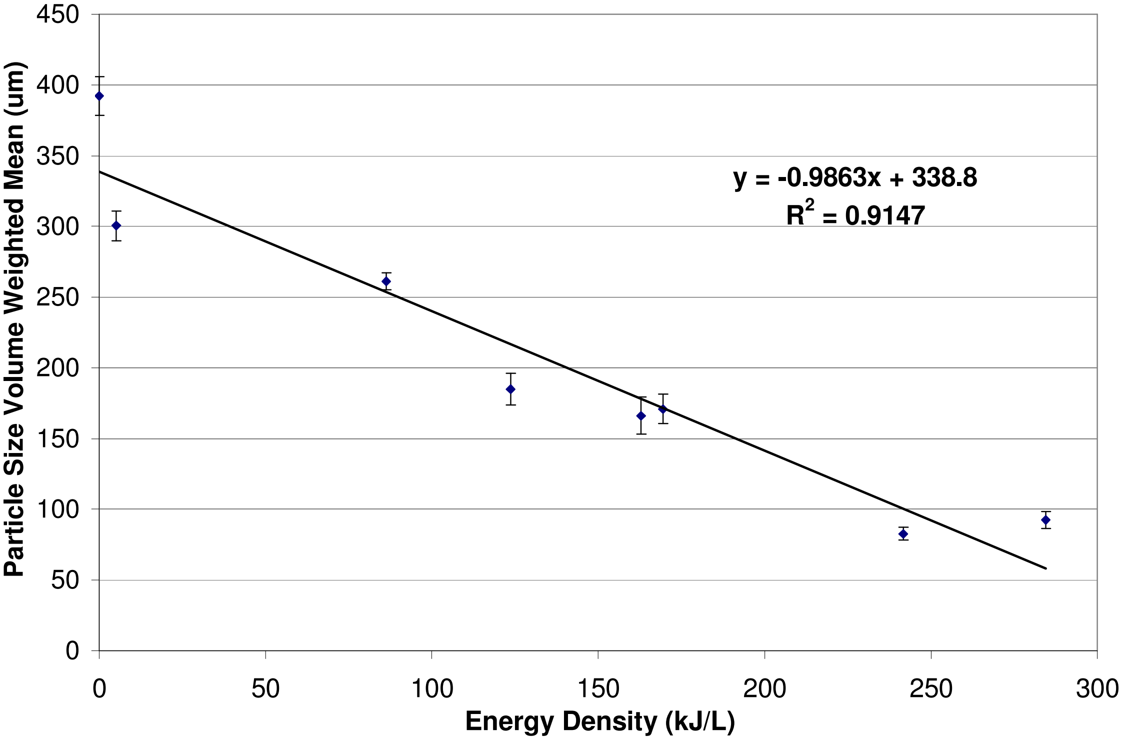


Figure 5.11 Effect of Ultrasonic Energy Density Introduced on the Particle Size Volume Weighted Mean of Corn Slurry

Conclusions

This study is a comparative investigation of batch and continuous flow ultrasonic modes in terms of their saccharification yield and particle size characterization. The batch ultrasonic mode resulted in higher yields of reducing sugar compared to the continuous flow mode. However, based on the energy density and net relative energy gain, the continuous flow ultrasonic system was more efficient. Both ultrasonic systems resulted in particle size reduction of corn slurry relative to the control (untreated). The morphology of the corn particle showed endorsperm fraction disintegrated into smaller clusters during sonication. It was also found that the particle size reduction was directly proportional to the energy density introduced. This study concludes that batch ultrasonic treatment can be very effective in small scale experiments. However, for large scale set-up, continuous flow ultrasonic system is recommended.

Acknowledgement

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CHAPTER 6: ULTRASONIC PRETREATMENT OF CORN FOR SIMULTANEOUS SACCHARIFICATION AND FERMENTATION IN ETHANOL PRODUCTION

A paper to be submitted to *Energy & Fuels*

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Abstract

The potential application of ultrasonics as a pretreatment process to enhance saccharification of starch in corn-to-ethanol plants is evaluated in this paper. Due to energy intensive use of steam in hydro-cooking, ultrasonics poses a promising alternative as a pretreatment method. Two independent ultrasonic experiments were conducted at a frequency of 20 kHz;batch and continuous flow treatment. Corn slurry was obtained from a nearby ethanol plant and sonicated in batch mode at an amplitude of 144µm$_{\text{peak-to-peak (p-p)}}$ for 90 seconds using a catenoidal horn. In the continuous flow treatment, corn slurry was pumped through a reactor using Branson Ultrasonic's "donut horn". Jet cooked samples were obtained from the same ethanol plant and analyzed for comparison of fermentation yield. The highest ethanol conversion was obtained by the jet cooked

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samples with a yield of 64% (relative to theoretical conversion). Similar results were obtained by the sonicated samples in both batch and continuous flow treatments at 61% and 58%, respectively. The glucose levels decreased with consumption during the initial stage of the fermentation then suddenly dropped after the 6th hour as the ethanol increased. The control samples (without any pretreatment – ground corn only) was only treated with enzymes to hydrolyze the starch to sugar had a slower ethanol production compared to the treated samples. An economic analysis was also conducted comparing jet cooking and ultrasonics pretreatment methods. The analysis showed that capital cost for the ultrasonics system was 15-times higher compared to the capital cost of hydrocooker. However, due to the relatively large energy requirements of hydrocookers, the operating costs of the hydrocooker suggest that it is still cost effective to use utlrasonics. In a 10-yr model, both pretreatment showed positive net present value indicating a good investment but ultrasonics treatment was 10% lower than the jet cooker. Yet, in terms of benefit cost ratio, ultrasonics treatment was found 25% higher than jet cooking. Therefore, this study has concluded that ultrasonics can be considered a promising alternative to jet cooking.

Keywords

Corn slurry, Ethanol, Lactic Acid, Acetic Acid, Glucose, Fermentation, Ultrasonics, Jet Cooking, Economic Analysis, Energy Gain

Introduction

Skyrocketing world oil prices and increasing global awareness of petroleum's negative environmental effects has increased ethanol demand. According to the U.S. Energy Information Administration [1], ethanol production has increased from 49 thousand barrels per day in 1990 to 425 thousand barrels per day in 2007. While fuel ethanol production has become slightly more efficient in recent years, additional technologies can improve the long-term sustainability and profitability of the industry.

Over the years, several studies have argued that ethanol should not be produced due to its negative net energy value (NEV) [2,3]. Net energy value (NEV) is defined as "the energy content of ethanol minus the fossil energy used to produce ethanol" [4]. Pimentel [5] indicated that 99,119 Btu (104.5 MJ) of energy is required to produce a gallon of ethanol, resulting in a net energy loss of 22,119 Btu (23.3 MJ)of energy per gallon, a value lower value than reported by others [4,6]. In contrast, Shapouri et al. [4] showed that a 34% net energy is gained from a gallon of ethanol. Farrell et al. [7] added that the net negative energy gain reported in the literature was due to the omission of co-products and use of some obsolete data in the calculations by Pimental [5]. Because of these contradicting publications, ambiguities in the energy balance measurements, and relatively small energy gain, any improvement in the efficiency of the production of ethanol would help secure its viability as a renewable energy source.

Jet cooking in typical dry-grind ethanol plants, where corn mash is mixed with steam, is one of the energy-intensive steps. The goal of this operation is to induce starch gelatinization and aid the enzymatic conversion of starch to monosaccharide units. In this process, the jet cooker uses steam at pressures of 1 MPa(150 psi) and temperatures ranging from 121 to 148 °C. In a state-of-the-art dry milling plant, production of 1 gallon of ethanol requires a thermal energy of 18,147 kJ (17,200 Btu) using natural gas or 33,129 kJ (31,400 Btu) using coal as fuel [8]. Because approximately 5% of this energy is used for jet cooking, enhancement in the efficiency of this process will result in improvements in the net energy balance of ethanol produced from corn.

Ultrasound is the range of sound waves at a frequency above the normal human hearing range (> 18-20 kHz). As ultrasound waves propagate in a liquid medium, cavitation occurs [9,10]. "Cavitation is the production of microbubbles in liquid when a large negative pressure is applied to it" [11]. Because of surface tension and the presence of other bubbles, foreign bodies, and gradients in the pressure waves, each bubble becomes unstable beyond a critical size and eventually collapses violently [11]. As the bubbles collapse, localized temperatures reaching up to 5000 K and pressures up to 180 MPa can be achieved [12,10]. In addition, because most bubbles collapse asymmetrically they produce very high shearing within the liquid which can fracture nearby solids, such as corn particles. Ultrasound waves in liquid media also produce streaming, which is used to facilitate the uniform distribution of ultrasound energy in the corn slurry, convection of the media, and dissipation of any heat produced [13,10].

Ultrasonic technologies have been studied in diverse industrial applications for decades. Zhang et al. [14] used ultrasound to enhance fractionation of yellow dent corn into its components (starch, germ, fiber, gluten). The study also showed higher starch separation and lower protein content in the resulting starch fraction. Several investigators also evaluated the use of ultrasonics to treat highly polymerized compounds [9,15,16,17]. In starch solutions, depolymerization of large molecules can occur as a result of ultrasonic treatment [18,17]. In these cases, cavitation breaks the bonds between the chain molecules and breaks them into smaller molecules. Lorimer et al. [19] confirmed this in the permanent reduction of relative molar mass of native dextran.

The use of ultrasound in enzymatic hydrolysis has also been explored. Studies have shown that at low ultrasonic power, some enzymes are not deactivated [20,10]. In a study by Wood et al. [21], ultrasonication (pulse mode) was found to increase ethanol yield in the simultaneous saccharification and fermentation (SSF) of mixed waste office paper. The study also showed that ultrasonics assisted experiments only require half the amount of enzyme to produce an ethanol yield similar to that produced without sonication. Furthermore, in the field of immobilized enzymes, ultrasonic application has been found to be very effective in increasing the activity of α-chymotrypsin on agarose gel [22]. Shah and Gupta [23] found similar positive findings in enzymatic activities and transesterification of lipases (from *Burkholderia cepacia* and *Pseudomonas fluorescens*) in an aqueous buffer. These finding suggests that ultrasonics can be an attractive and cost-effective method to reduce enzyme use in various applications.

In a fermentation facility, bacterial contamination can reduce the overall efficiency of a given process. To reduce bacterial contamination, one task of jet cooking is to sterilize the corn mash before fermentation. Ultrasound has also been known to break down biological cells. In more detail, destruction of bacteria with ultrasonics combine with chemical treatment has been studied as an alternative method to conventional sterilization (steam treatment or dry heat) [24]. Even though complete destruction of bacteria may be difficult with ultrasonics treatment alone, yet it may have the advantage of shorter operating time and use of lower temperatures than conventional sterilization methods [25]. In the environmental engineering field, ultrasound has been used to induce cell disruption in waste activated sludge (WAS) for improved anaerobic digestion [10,26].

Based on the findings of others, there is a strong potential that ultrasonication can be used as a pretreatment method to enhance ethanol production. Thus, the objectives of this study are to determine the effects of ultrasonication in the fermentation yield and evaluate the cost effectiveness for full-scale application.

Materials and Methods

Corn Samples

Corn slurry samples were obtained directly from slurry tanks and hydrocookers of a nearby ethanol plant (Lincolnway Energy, Nevada, IA). Figure 6.1 shows the schematic diagram of the prefermentation steps in this typical dry grind ethanol plant. Whole corn kernel is delivered to the plant, where it is ground by hammer mills to a specified particle size distribution. Ground corn is mixed with cooked water (backset, CO_2 scrubber water & recycled process water from evaporator condensate) and steam to form the slurry with temperature reaching 82-85°C. This step is often called mashing. After mashing, one-third of the required alpha amylase enzyme is added to the mash. It is then pumped to the hydro cooker, where it is exposed to steam with temperatures ranging from 121 to 148°C and a pressure of 0.689 MPa (100 psi). The slurry is immediately pumped to the liquefaction tank, where the balance of the alpha amylase necessary is added. The temperature and retention time of the liquefaction tank was 82-85°C and 90 minutes, respectively.

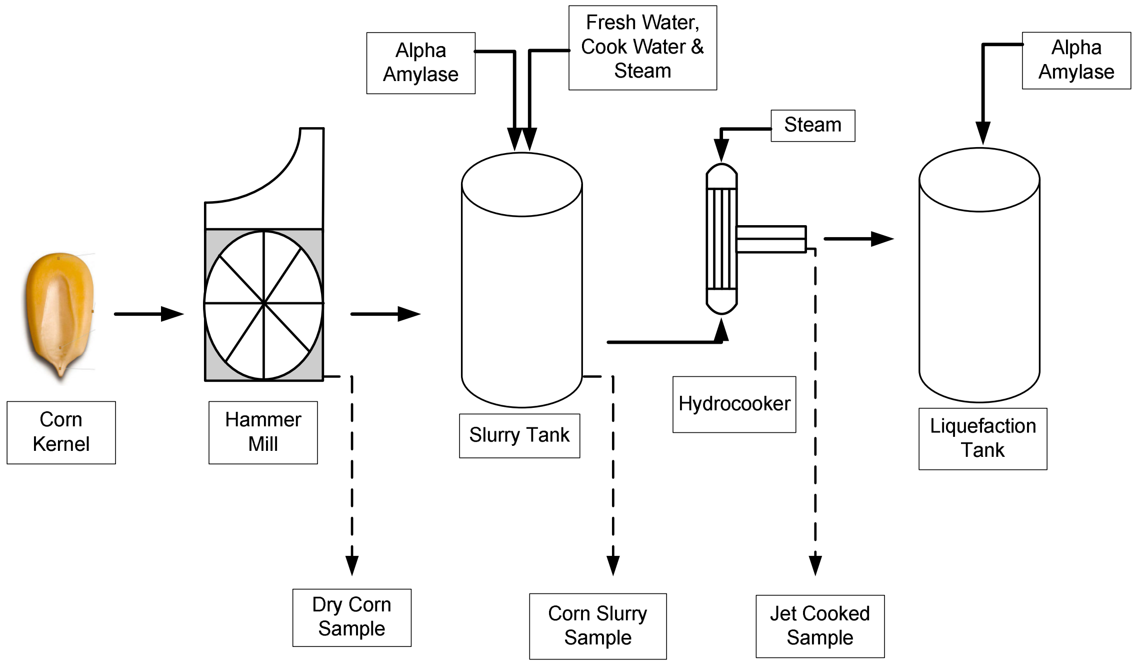


Figure 6.1 Schematic Diagram of Pre-Fermentation Treatment in a Typical Dry Grind Ethanol Plant

In this study, three types of samples were taken from the ethanol plant, dry ground corn, corn slurry from the slurry tank and jet cooked corn slurry (Figure 6.1). Dry ground corn mixed with water represents the control where no pretreatment was done. Corn slurry was taken from the slurry tank and sonicated in batch and continuous flow mode. While corn slurry sample and jet cooked corn sample were used directly for fermentation. The enzyme used in this study was STARGEN^{TM} 001 (456 granular starch hydrolyzing units in GSHU/g) from Genencor International (Palo Alto, CA). The composition of the corn was determined using a Near-Infrared (NIR) Infratec^{TM} 1241 Grain Analyzer (FOSS Tecator, Eden Prairie, MN).

Batch Ultrasonic Experiment

Corn slurry samples (50ml) from the slurry tank were mixed with 50ml sterile distilled water and sonicated for 90s at an amplitude of $144\mu m_{(p-p)}$. The batch experiment was conducted in a Branson 2000 Series bench-scale ultrasonic unit (Branson Ultrasonics, Danbury, CT) using a catenoidal horn. The horn was a standard 20 kHz half-wavelength catenoidal titanium with a flat 13 mm diameter face (gain = 1:8). The system has a maximum power output of 2.2 kW and a frequency of 20 kHz. The average ultrasonic energy dissipated into the sample is presented in Table 6.1.

Table 6.1 Average Ultrasonic Energy in Batch and Continuous Flow Experiments

	Average Ultrasonic Energy (J)	**Average Ultrasonic Density Dissipated (kJ/L)**	**Temperature change due to Ultrasonication**
Batch Ultrasonics	12,000	120	26 °C ± 6.1
Continuous flow Ultrasonics	230	4	none

Ultrasonic Continuous Flow Experiments

Continuous flow experiments were conducted using Branson 2000 Series bench-scale ultrasonic unit (Branson Ultrasonics, Danbury, CT) operating at 3 kW and frequency of 20 kHz. Corn slurry samples obtained from the ethanol plant was pumped from a continuously stirred feed tank to an ultrasonics reactor where the Branson Ultrasonics "donut" horn was installed (Figure 6.2). Reactor volume and retention time were 5.5L and 10s, respectively. The maximum volumetric flow rate of the pump was used 31.4 L/min (8.3 gal/min). A constant amplitude of 12 μm_{p-p} was maintained.

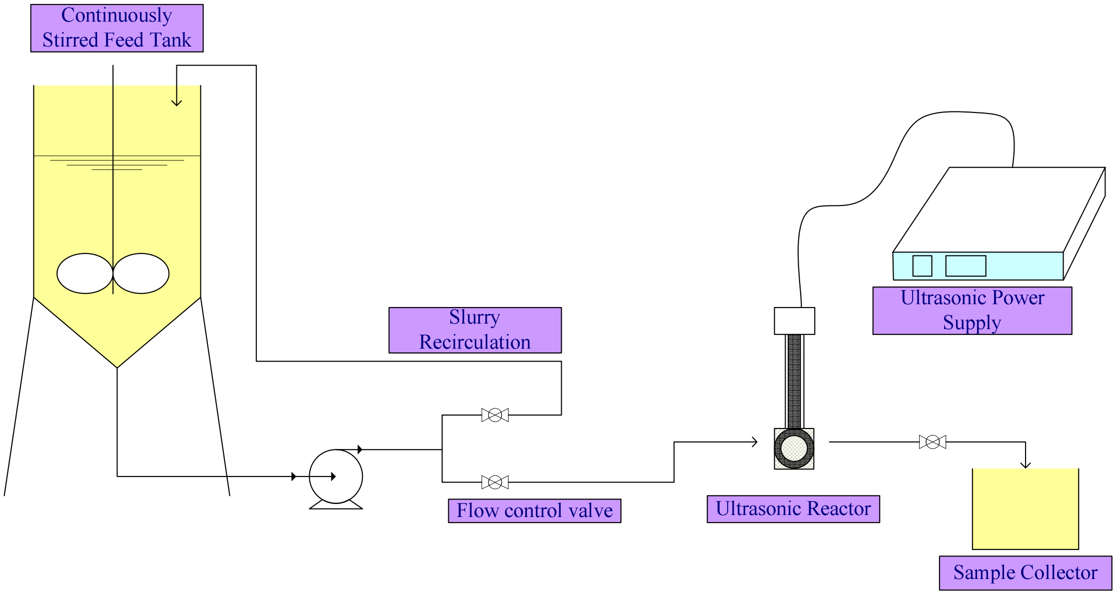


Figure 6.2 Continuously Flow Ultrasonic System Process Diagram

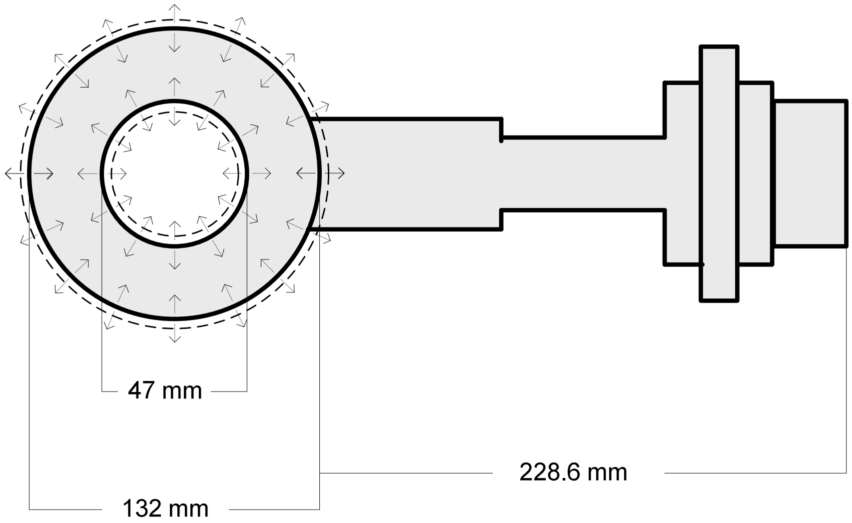


Figure 6.3 Branson Ultrasonics "Donut" Horn

Figure 6.2 & Figure 6.3 shows the schematic of the continuous flow experimental set-up and the donut shaped horn. It is seen that in order to assure proper mixing and reduce fouling of the pump, the corn slurry was continuously mixed in the feed tank as well as recirculated using the pump. Figure 6.3 shows the donut shaped structure of the ultrasonic horn. As seen in the figure, the horn vibrates in radial direction. The horn was placed in vertical position inside a closed reaction chamber where fluid flows primarily through its center with some flow around it.

Simultaneous Saccharification and Fermentation (SSF) Experiment

The 48-h fermentation was carried out in 250ml centrifuge bottle at 32°C shaking at 180 rpm. The experiment was a modified protocol based on NREL Lap 008 [27]. Fermentation media (10x YP (Yeast extract-Peptone)) contained 100g/L yeast extract, 200g/L peptone and 1M citrate buffer (pH 4.3) [27]. Excess nutrient media was provided, thus it was not a limiting factor. The propogated yeast was prepared by adding 100mg of dry industrial yeast (Lincolnway Energy, Nevada, Iowa) to the propagation media containing 100 ml of YP with 5% glucose [27], 40 ml distilled water and 10ml 1M citrate buffer (pH 4.3). The broth was fermented in shaking incubator at 32°C for 18-20 hours. YP with 5% glucose media was composed of 50g/L glucose, 20g/L peptone and 10g/L yeast extract. All fermentation media were sterlized in an autoclave at 121°C for 15 minutes. Figure 6.4 details the fermentation conditions included in the study and Table 6.2 details the various terms used.

A reference sample was fermented in parallel with all experiments, which contained 10ml 10x YP media, 10ml 1M citrate buffer (pH 4.3), 10ml propagated yeast innoculum, 80μl Stargen enzyme and 120ml sterile distilled water. Because yeast was propagated in a media containing 5% glucose and due to Crabtree effect [28, 29], it is expected that trace amounts of ethanol during propagation period would be produce which would result in experimental error. In addition, residual glucose in the propagated yeast innoculum would add to the total amount of sugar in the fermentation experiments. In order to account for these experimental errors, the amount of ethanol obtained in the reference sample was deducted to the amount of ethanol produced during SSF experiments.

Table 6.2 Definition of Terms used as Fermentation Conditions

Term	Definition
Control	Fermentation sample containing ground corn with enzyme and yeast added
Corn Slurry	Corn slurry sample obtained from ethanol plant with enzyme and yeast added
Jet Cooked	Jet cooked sample obtained from ethanol plant with enzyme and yeast added
Batch Ultrasonics	Corn slurry sample obtained from ethanol plant sonicated in batch mode using catenodal horn with enzyme and yeast added
Continuous Flow Ultrasonics	Corn slurry sample obtained from ethanol plant sonicated in continuous flow mode using the “donut” shaped horn with enzyme and yeast added

Analytical Method

During the experiment, 5ml samples were aseptically taken at 0, 6, 12, 18, 24, 48 hours of fermentation. In every sampling period, the fermentation broth was centrifuged at 3000 rpm for 10 minutes to exclude corn particles in the sample. The samples were placed in a sealed container, then immersed in a boiling water bath for 10 min to denature the enzyme and inactivate the yeast cells. Samples were cooled in ice bath and analyzed for glucose using a modified dinitrosalicylic acid (DNS) method [30,31]. Ethanol, lactic acid and acetic acid were analyzed using high performance liquid chromatography with refractive index detector (HPLC: Varian ProStar 210, MetaCarb 87P column with mobile phase of 0.01N Sulfuric Acid, flow rate of 0.6ml/min, column temperature of 80°C and injection volume of 20µl).

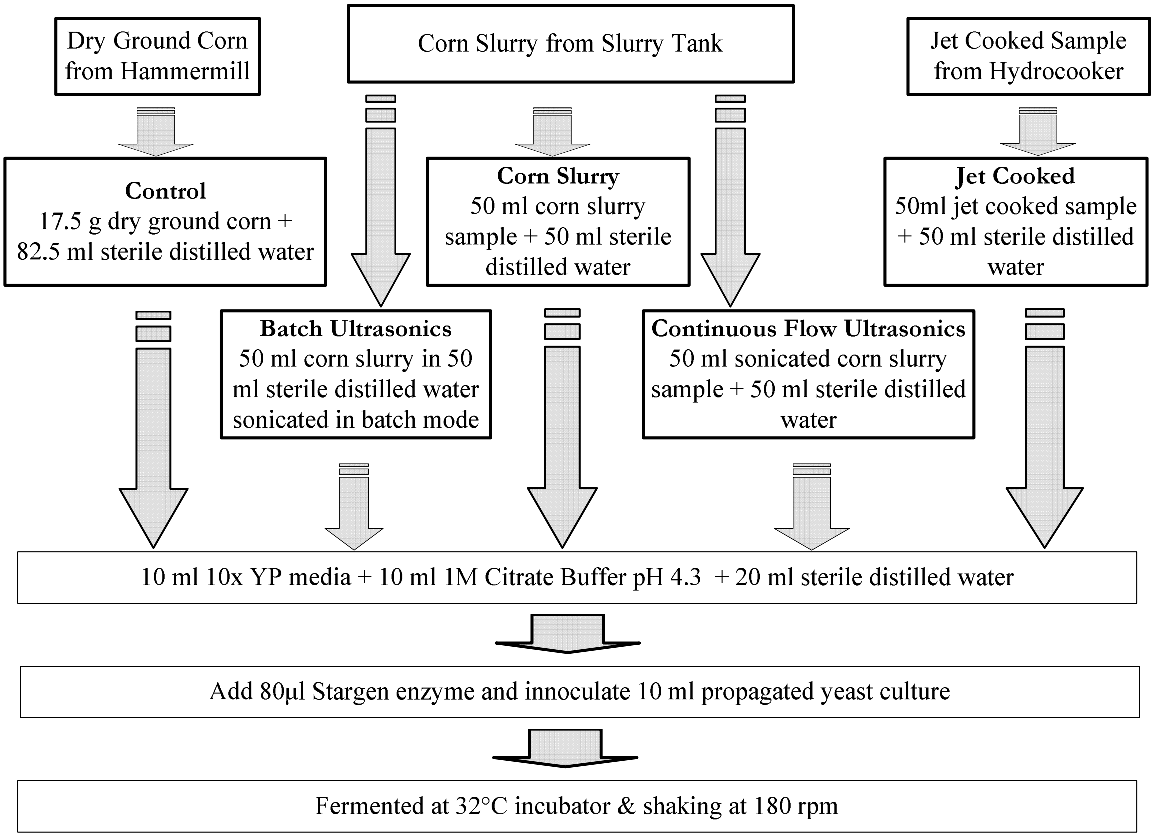


Figure 6.4 Overview of the Simultaneous Saccharification and Fermentation Conditions

Yeast cell count was conducted using hemacytometer microscope method [32, 33]. All budding and clumped or clustered yeast cells were counted as one cell when this method was used. Dilutions were also made to high density yeast cells samples. Total solids concentration was also analyzed in the study [34]. The drying temperature used was 45°C to avoid gelatinization of starch. All experiments and analytical procedures were conducted in duplicate and triplicate, respectively. Statistical analysis was done using Statistical Analysis Software (SAS) 9.1 for Windows.

Ethanol conversion based on theoretical yields were calculated in Equation 6.1 assuming a maximum 51.1 g ethanol production per 100 g of glucose consumed by yeast. The initial amount of glucose was based on the starch content of corn. The composition of ground corn was found to be 7.6% protein, 73.1% starch, 2.6% oil and 16.7% moisture.

$$Ethanol\ conversion\ (\%) = \frac{ethanol\ in\ sample\ (g) - ethanol\ in\ ref.\ (g)}{initial\ amt.\ glu\cos e\ \ from\ starch\ (g) \times 0.511} \quad \times \quad 100$$

----------- [Eqn 6.1]

Results and Discussion

Simultaneous Saccharification and Fermentation Yield

Figure 6.5 shows the ethanol production and glucose consumption of yeast using corn as substrate at varying pretreatment conditions. During the first 6 hours of fermentation, the glucose concentration continued to increase then suddenly dropped to less than 4g/L at the 12th-h sample signifying the yeast's glucose consumption. Subsequently, ethanol yield only started to increase after 6 hours of fermentation. The first 6 hours is the lag phase or the adaptation period of the yeast [35]. It is believed that the ethanol detected during lag phase is due to the propagation step which reduced the lag phase [35].

In Figure 6.5, the two highest ethanol yields was obtained by the samples sonicated in batch and jet cooked sample: 38.72 g/L and 37.75 g/L, respectively. Similarly, both of these treatment conditions also had the highest glucose concentration during the first 6 hours of fermentation. This is the result of the additional sugar released during gelatinization of starch obtained both in jet cooking and ultrasonication pretreatment. Because continuous flow treatment has lower amplitude and power input than batch, it did not gelatinize the starch as the sugar concentration (40g/L) at time 0 was similar to corn slurry (Figure 6.5 A & D).

In contrast, the ethanol production and glucose consumption of the control (without treatment) showed significantly different results. It is observed that the glucose did not increase above 4g/L and started to decrease further at the 12th hour of fermentation. It is important to note that despite the low glucose concentration shown in Figure 6.5E, the yeast was able to produce a significant amount of ethanol (27g/L). It is believed that the available sugar that was produced by enzymatic hydrolysis was immediately consumed, thus it was not detected between the sampling period.

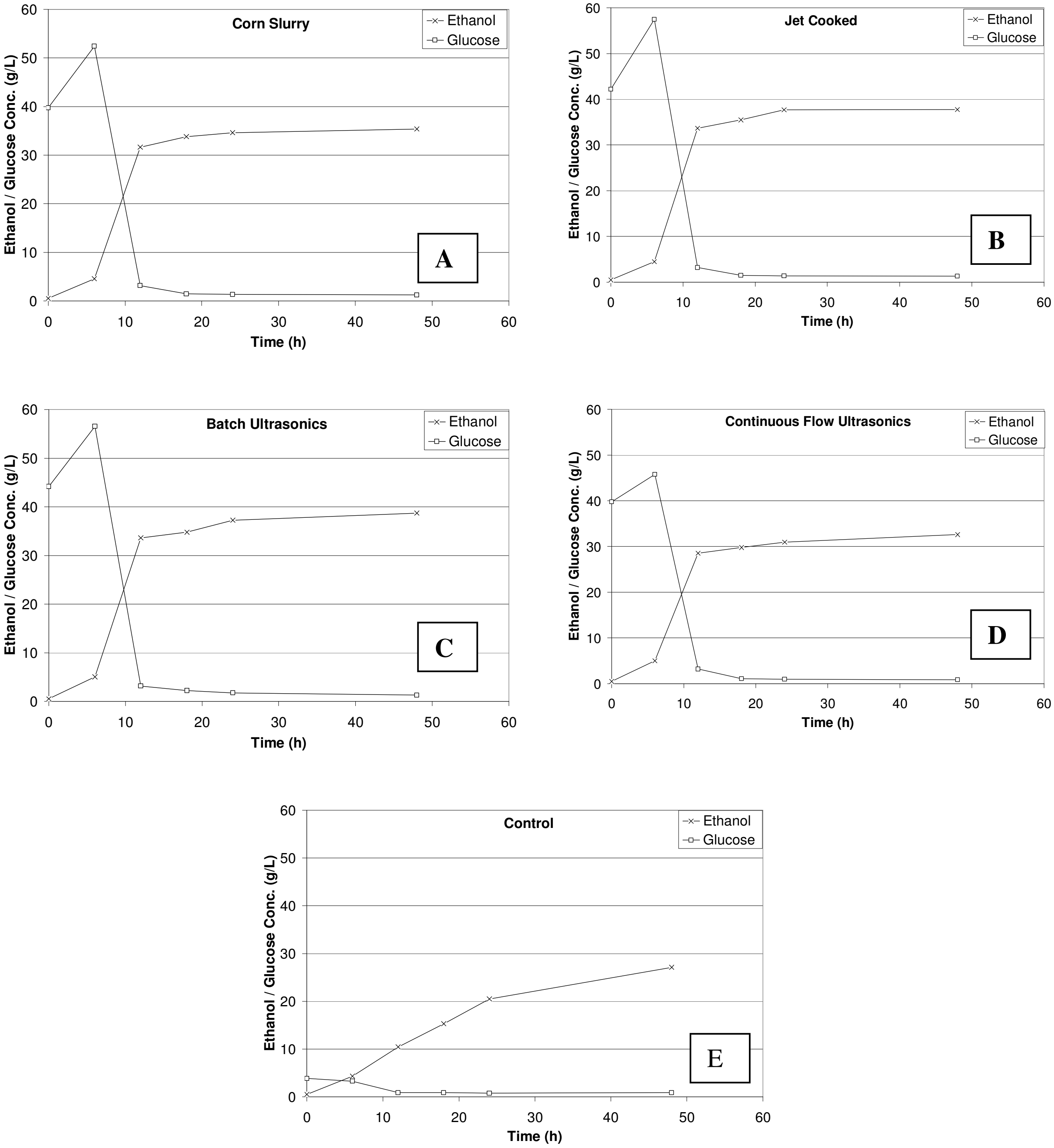
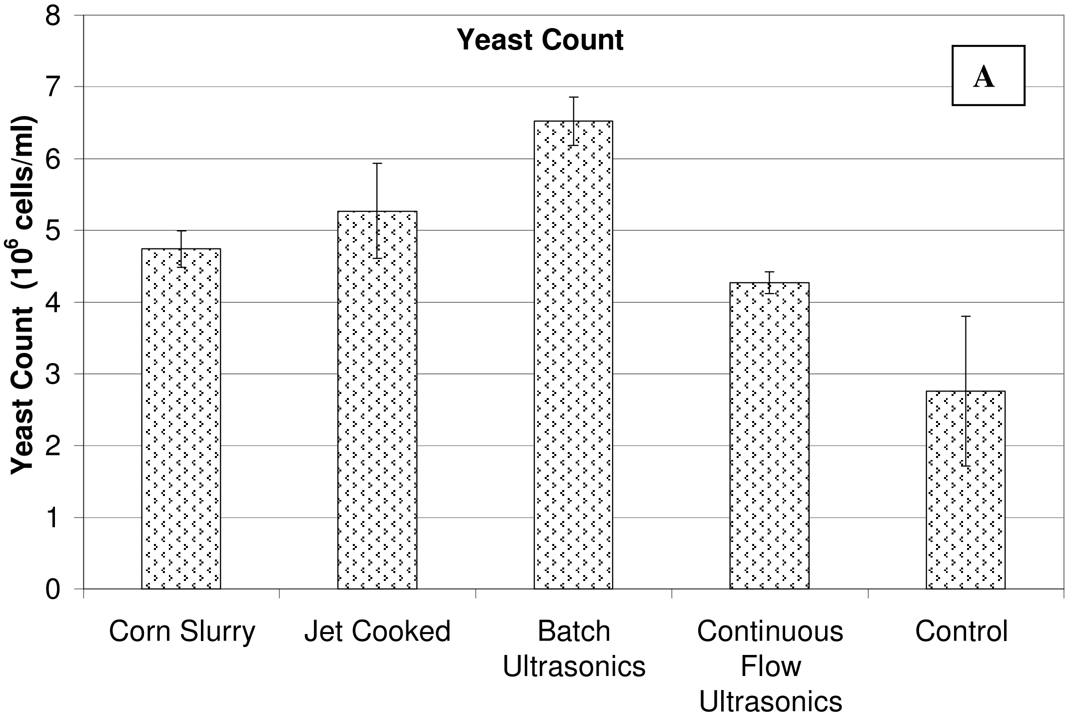


Figure 6.5 Ethanol Production and Glucose Consumption at Varying Treatment Conditions in 48h Fermentation: (A) Corn Slurry, (B) Jet Cooked, (C) Batch Ultrasonics, (D) Continuous Flow Ultrasonics, (E) Control


Yeast Count
A
Yeast Count (10^6 cells/ml)
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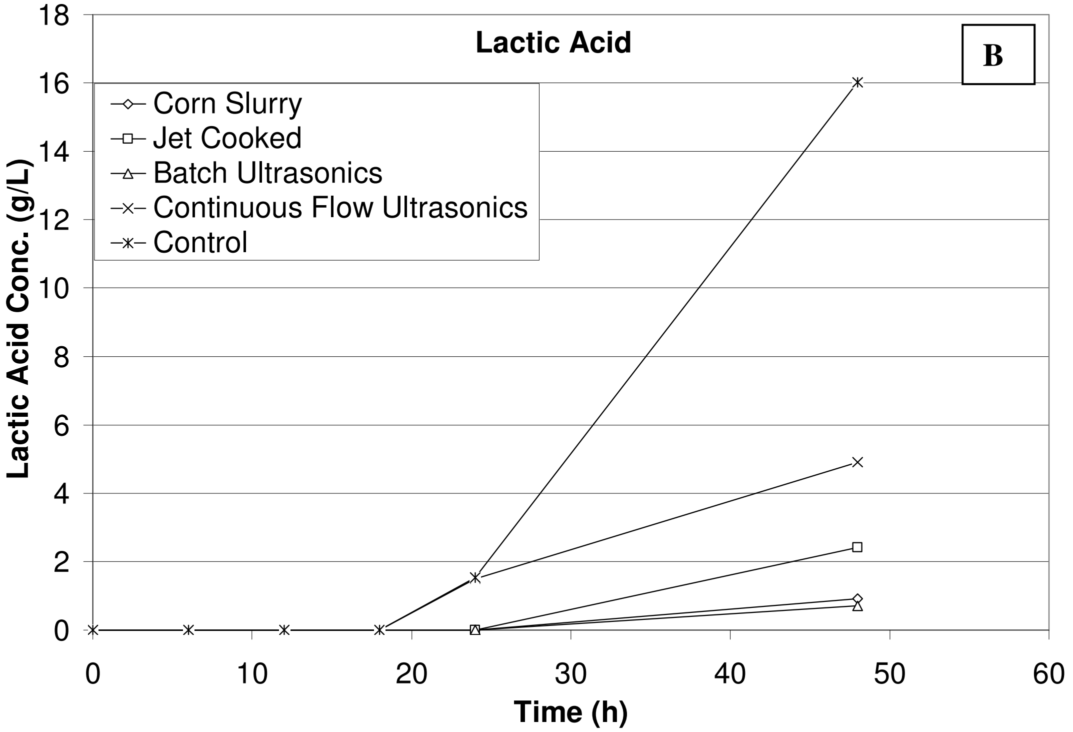
1
2
3
4
5
6
7
8
Corn Slurry
Jet Cooked
Batch Ultrasonics
Continuous Flow Ultrasonics
Control


Lactic Acid
B
Corn Slurry
Jet Cooked
Batch Ultrasonics
Continuous Flow Ultrasonics
Control
Lactic Acid Conc. (g/L)
0
2
4
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10
12
14
16
18
0
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30
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50
60
Time (h)

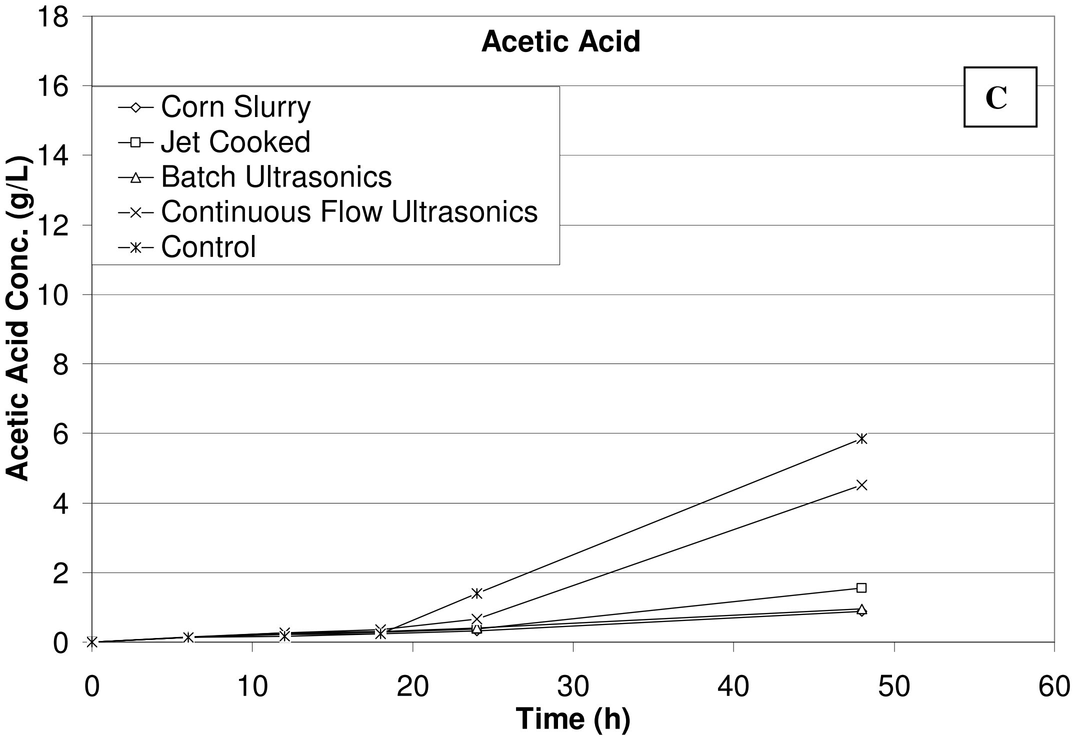

Figure 6.6 Yeast Cell Count (A), Lactic Acid (B), Acetic Acid (C) Production at Varying Treatment Condition in 48h Fermentation

Figure 6.6 shows the yeast count, lactic acid and acetic acid concentration with a 48h fermentation period comparing the various pretreatment conditions. In Figure 6.6A, the yeast count was obtained at the end of the fermentation (48th hour). It was found that sample sonicated in batch mode and jet cooked resulted in the highest biomass production, which also corresponded to their higher ethanol yields. However, comparing the ethanol yield and biomass produced by the two treated samples (batch ultrasonics and jet cooked), it is believed that the sonicated samples had more fermentable sugar than the jet cooked because both samples reached approximately the same ethanol yield. Similarly, corn slurry sample was found to have higher biomass concentration than the sonicated sample (continuous flow), yet both corn slurry and samples sonicated in continuous flow yielded comparable theoretical ethanol conversion (Figure 6.7).

In Figure 6.6B, the control (untreated) sample had the highest lactic acid concentration (16 g/L). In contrast, samples sonicated in batch has 1g/L of lactic acid. A similar trend was seen in acetic acid production. As seen in Figure 6.6C, the acetic acid produced was 5.9 g/L for the control sample. Similar to the continuous flow sample with a concentration of 4.5 g/L. A possible contamination was the initial finding for the high lactic acid concentration in the control sample. Yet, due to the very low glucose concentration in the control sample, bacterial propagation and survival maybe difficult. The bacteria, if assumed present, would also have to compete with the yeast for food. Futhermore, a microscope inspection showed no sign of lactic acid nor acetic acid producing bacteria in all the samples. Thus, it is believed that the yeast produced the acetic acid during fermentation. According to Piskur, et al. [36], the yeast has the ability to rapidly produce ethanol to inhibit the growth of competing microorganism, then eventually use the accumulated ethanol later when glucose depletes. The accumulated ethanol is then converted back to acetaldehyde via alcohol dehydrogenase enzyme and later into acetate. Thus, yeast produced acetic acid. *Saccharomyces cerevisiae* (yeast) is also known to utilize lactate when it is available in the fermentation medium [29]. Therefore, it is also possible that yeast could have produce lactic acid. However, because of insufficient evidence, the study recommends further investigation regarding this issue.

Figure 6.7 shows the ethanol conversion on theoretical yield of varying treatment conditions at 48-hour fermentation. The maximum theoretical ethanol yield (55.7-63.2 g/L) was calculated for each treatment conditions based on the initial starch content of each sample. The total solids concentration was found to vary relative to the sample. Corn slurry and samples sonicated in batch had a total solid concentration of 36% (m/v) while jet cooked sample was 34% (m/v). Samples sonicated in continuous flow had total solids concentration of 32% (m/v). A conversion ratio of 51.1 g of ethanol per 100 g of glucose was assumed. The 48th hour was selected as the completion point of fermentation.

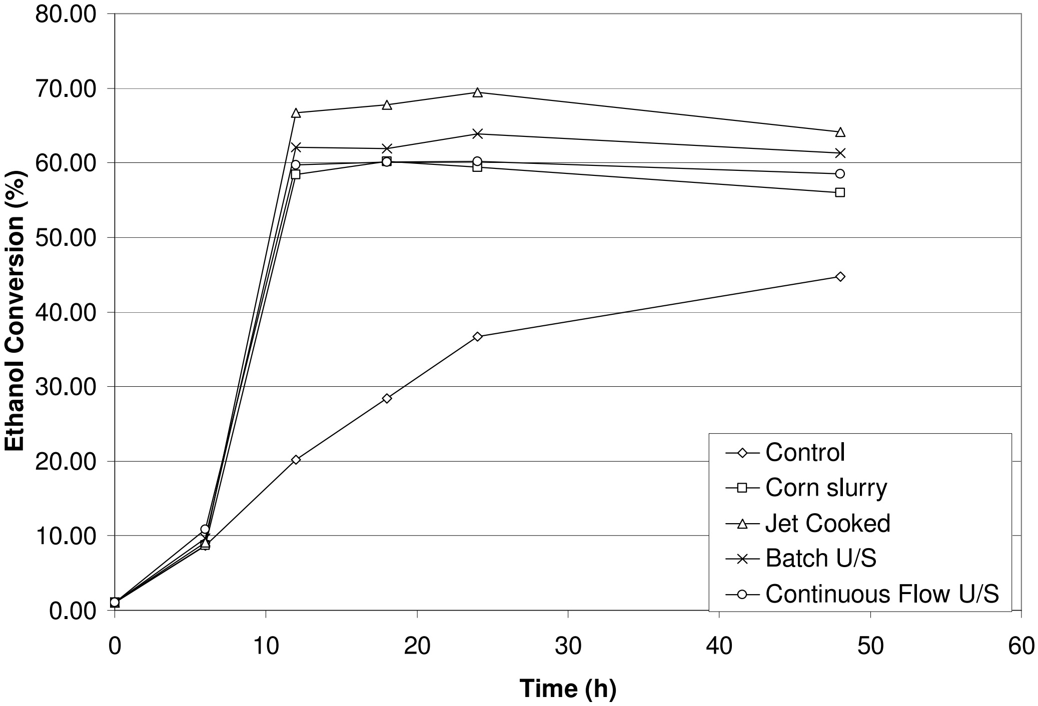

Figure 6.7 Ethanol Conversion based on Theoretical Yield at Varying Treatment Conditions in 48h Fermentation

It was seen in Figure 6.7 that maximum ethanol conversion ranged between 44.8 g/L (control) to 64.1 g/L (jet cooked). The highest ethanol conversion obtained by jet cooked samples was 69.5%, which corresponds to 2.5 gal/bushel conversion. For samples sonicated in batch & continuous system, the highest ethanol conversions were

63.9% and 60.1%, respectively. This corresponds to a conversion of 2.3 gal/bushel & 2.1 gal/bushel for batch and continuous flow systems, respectively. After the 24th hour, the theoretical ethanol conversion of the treated samples (corn slurry, jet cooked, and ultrasonication) decreased. The decrease in ethanol concentration was attributed to the ethanol consumption by yeast (discussed earlier), which was similar to results found by Isci, et al. [37]. Jet cooked samples obtained the highest ethanol conversion followed closely by the sample sonicated in batch and continuous flow. The difference in ethanol conversion between the jet cooked sample and the sonicated samples were 4.7% for the batch and 9.7% for the continuous flow. Similarly, a 4.4% difference was found between samples sonicated in continuous flow mode and the corn slurry sample. However, statistical analysis (P-value = 0.4113 at 95% confidence) showed no significant difference between the theoretical ethanol conversions of jet cooked and the sonicated samples. Even though the continuous flow ultrasonic system obtained lower ethanol conversion than the jet cooked sample, it is important to note that in a large scale system, more donut horn units will be used in the system. Thus, it is expected that efficiency will be enhanced.

Economic Analysis

To investigate the potential use of ultrasonics as pretreatment, a simple economic analysis was conducted. The objective of this analysis was to compare the current pretreatment technology (jet cooker) and the proposed pretreatment of this study (ultrasonics). Because continuous flow ultrasonics using the donut horn has been used in various large scale applications, it will be the ultrasonic method assumed in this analysis.

The economic analysis was based on the biobased economic analysis of Brown [38]. While this study focuses on the prefermentation treatment of ethanol plants, it will only compare the direct, indirect and operating cost of the two treatment methods.

Table 6.3 summarizes the assumptions of the economic analysis. Ethanol dry grind plants operate 24h/day, year round with predetermined shutdown time (15 days) for maintenance. A basis of 350 days per year (8400h) operating time was used in this analysis. The ethanol plant capacity was based on 189 million L (50 million gallons) per year. Material module factor (MMF) is defined as the ratio of the cost of materials to install a particular piece of equipment to the cost of the equipment. Labor module factor (LMF) is the ratio of the cost of labor to install a particular piece of equipment to the combined cost of the installed equipment and materials used to perform the installation. A tabulated MMF and LMF for common industrial equipments were defined by Brown [38]. However, both hydrocooker and ultrasonics were not included in the list, thus an MMF and LMF of 0.27 was assumed. Operating labor cost was not considered in the computation because it only requires a fraction of the operator's work coverage. Steam and electricity were used to account for the utility costs of jet cooker and ultrasonics, respectively. All assumptions were converted to present (2008) dollar value using inflation index [38].

Because there is insufficient public information on performance of hydrocookers, the values in Table 6.3 for jet cooking were obtained from private ethanol industries [41]. The total hydrocooker cost included steam flow control and temperature control. The cost of steam in Table 6.3 was obtained from Kwiatkowski, et. al [40] while steam flow rate and approximate hydrocooker cost were based on statistical data of a typical dry grind milling plant [41].

Table 6.3 Assumptions of the Economic Analysis in 2008 Dollars

	Jet Cooker	**Ultrasonics**
Annual Production (gal/yr)	50,000,000	50,000,000
Annual operating hours	8400	8400
Material module factor (MMF)	0.27 [38,39]	0.27 [38,39]
Labor module factor (LMF)	0.04	0.04
Steam cost ($/1000kg)	17.93 [40]	-
Steam flow rate (lb/h)	10,000 [41]	
Electricity cost (cents/kW-h)	-	6.68 [42]
Number of ultrasonics unit to be installed	-	85
Ultrasonics power required (W/unit)	-	3,000
Maintenance and repair (% of capital cost)	4%	10%
Assumed approximate cost of jet cooker ($)	52,737	-
Ultrasonics cost ($/unit)	-	10,000
Interest rate, i	15%	15%

On this benchscale study of the ultrasonics continuous flow system, the highest flowrate achieved was approximatety 26.46 L/min (7 gal/min). If a maximum flowrate in a full-scale plant reaches 2268 L/min (600 gal/min), approximately 85 ultrasonics donut horn units are needed to maintain similar retention time. Ultrasonic unit cost was estimated and the industry electric utility cost were obtain from the US energy information administration [42]. The maintenance and repair assumptions were assumed on a percentage scale from the capital cost [38]. However, it is known that ultrasonic horn will wear when continuously operated, therefore maintenance and repair for ultrasonics was set at 10% while jet cooker was set at 4%.

Table 6.4 Comparative Economic Analysis of Continuous Flow Ultrasonics and Jet Cooking as Pretreatment

Fixed Capital Cost		**Jet Cooker (2008) $**	**Ultrasonics (2008) $**
Direct Cost			
Equipment, C_p		52,737	850,000
Materials for installation, C_m	MMF x C_p	2,637	42,500
Direct installation labor, C_l	LMF x ($C_p + C_m$)	14,951	240,975
Total Direct Cost, C_d	$C_p + C_m + C_l$	**70,324**	**1,133,475**
Indirect Cost			
Freight, insurance, taxes, C_{fit}	0.08 x C_p	4,219	68,000
Construction overhead, C_o	0.7 x C_p	10,466	168,683
Eng'g expenses, C_e	0.15 x (C_p+C_m)	8,306	133,875
Total Indirect Cost, C_{id}	$C_{fit} + C_o + C_e$	**22,991**	**370,558**
Fixed Capital Cost, C_f	$C_{id} + C_d$	**93,311**	**1,504,033**
Annual Capital Charges (annual payment to bank loan)	$C_{capital}$	**18,593**	**299,682**
Operating Cost			
Utilities		684,781	140,575
Maintenance & repair		3,733	150,403
Total annual operating cost	$C_{operating}$	**688,514**	**290,978**
Annual pretreatment cost	$C_{operating} + C_{capital}$	**707,107**	**590,660**
Product cost (cents/gal) due to pretreatment	Annual production cost / annual production	**1.4**	**1.1**

Table 6.4 shows the summary of the economic analysis comparing jet cooking and ultrasonication method. The total capital cost was detailed into two catergories: direct expenses and indirect expenses. "Direct expenses include the purchase price of the equipment, cost of materials required for installation and salary for installation labor" [38] while indirect cost were classified as "freight, insurance and taxes; construction overhead and engineering expenses" [38]. The indirect costs were the expenses related to

the installation of the equipment but were not included in the calculation of direct cost. In U.S., freight, insurance and taxes can be estimated as 8% of the total equipment cost (C_p) [38]. However, construction overhead (C_c) included fringe benefits on labor, construction machinery cost, and site clean up cost which can be as high as 70% of the labor cost (C_l) [38]. The engineering costs were the salaries and benefits for design engineers, office supply expenses and associated overhead, which was assumed to be 15% of the combine equipment (C_p) and installation materials (C_m) cost [38].

The total pretreatment cost was the sum of the annual operating cost ($C_{operating}$) and annual capital charge ($C_{capital}$). Annual capital charge ($C_{capital}$) is the cost a company has to pay the bank if a fixed capital cost was secured through a loan with an annual interest rate of 15% over a payment period of 10 years. The annual pretreatment cost was then divided by 189 million liters (50 million gallons) to determine the pretreatment cost per gallon of ethanol produced.

In Table 6.4, the total capital costs was estimated to be \$93,315 and \$1,504,033 for the jet cooker and ultrasonics, respectively. Assuming this funding was secured from a bank loaned, only the capital charge was considered in the calculation. The annual capital charges were \$18,593 for the jet cooker and \$299,682 for the ultrasonics. Despite that the ultrasonic's capital cost was more than 15-times the cost of the hydrocooker, the operating cost for jet cooking was found to be 5-times more expensive compared to the ultrasonics. By considering only the annual capital charge and the operating cost, it is seen that the modeled plant would save approximately \$116,447 each year on pretreatment cost if it invested in ultrasonics.

To further evaluate the economic feasibility of the pretreatments, net present value and cost-benefit ratio were assessed [43]. Net present value (NPV) is an indicator of the value an investment adds to the value of the company [44]. In order to determine the most profitable option, benefit cost ratio (BCR) is a method which weighs the total expected benefits against the total expected cost [45]. Assuming the same ethanol price for both pretreatment methods and taking into account the inflation rate [46], the annual rate of return for the plant using ultrasonics would be 2.5% compared to 2.1% for the plant using a jet cooker. It is important to note that the selling price ($0.03/gallon) assumption is based only on the pretreatment cost. Additionally, with the discount rate of 10% for a period of 10 years, the net present value of ultrasonics is $4.7M while jet cooker is $5.2M. In terms of benefit cost ration, using ultrasonics is 25% higher than jet cooker. These results indicate that investing in ultrasonics is more cost-effective compared to investing in the jet cooker.

Figure 6.8 shows the annual production cost of ultrasonics and jet cooker as a function of the number of ultrasonics unit installed. Using the same economic analysis as previously detailed, the assumption of ultrasonics unit was varied, thus obtaining the various annual production cost for ultrasonics pretreatment. As seen, as the ultrasonics unit increases, the annual production cost also increases linearly. Because the calculation for equipment cost and utility cost is proportional to the number of ultrasonics installed, thus it is expected that the increase is linear. Similarly, comparing the annual production cost of ultrasonics with a jet cooker, as the number of ultrasonics unit increases, the production cost difference between the two treatments also decreases. It is observed that if the number of ultrasonics unit installed is greater than 155, the two pretreatment's annual production cost are similar. However, it is recommended that experiments with multiple number of ultrasonics unit and higher flowrates should be conducted to evidently confirm the relationship between the ultrasonics efficiency with the number of ultrasonics unit that needs to be installed.

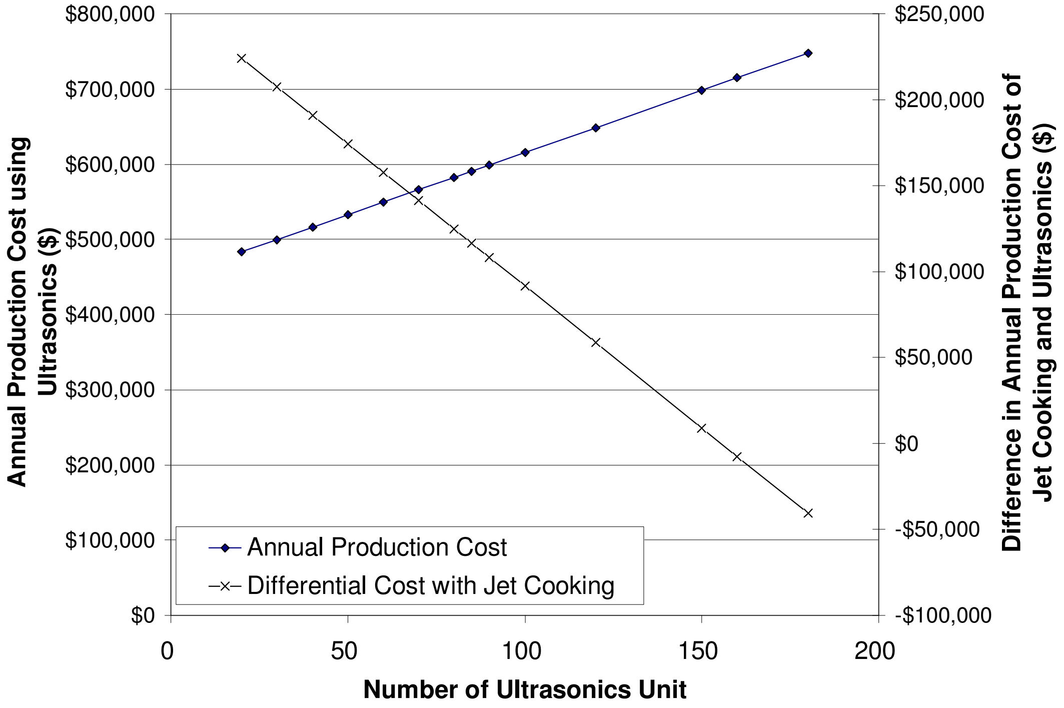

Figure 6.8 Annual Production Cost of Ultrasonics and Jet Cooker as a Function of Ultrasonics Unit Installed

Conclusions

This study was an evaluation of the potential application of ultrasonics as an alternative pretreatment method to jet cooking. There were 5 types of sample considered in the study; control (non-treated), corn slurry (partially treated in the plant), batch ultrasonication, continuous flow ultrasonication and jet cooked (obtained from ethanol plant). The glucose consumption in most treated samples reached a peak and immediately dropped as the ethanol concentration starts to increase. However, with the untreated control group, sugar was produced slowly thus it was consumed immediately as it becomes available. The highest ethanol conversion was achieved by the jet cooked samples followed closely by the samples sonicated in batch and continuous flow mode. Statistical analysis indicated no significant difference between jet cooked and ultrasonicated samples. Lactic acid and acetic acid were also produced during fermentation in all samples. However it was found predominantly in the control and samples sonicated in continuous flow. Because no bacterial contamination was detected in these samples, it was concluded that the yeast produced the acetic acid. For lactic acid production, the reason for its production was unknown and it is recommended for further investigation.

A simple economic analysis was also conducted to compare the two treatments methods (ultrasonication and jet cooking). Based on a system design using the donut horn, the continuous flow ultrasonic system was assumed as the treatment point of comparison. It was found that installing ultrasonication in the plant would cost significantly more compared to installing a hydrocooker. However, considering the annual capital charge amount (sum paid to the bank when capital cost was loaned) and the operating cost, ultrasonics was found to be a more cost effective investment compared to jet cooking.

Based on the observation that ultrasonic pretreatment performs as well as conventional jet cooker and the total cost are lower for ultrasonic treatment, it is concluded that ultrasonics can be considered a promising prefermentation treatment for corn ethanol plants.

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CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

The unstable cost of petroleum and vulnerability of foreign oil supplies to the United States, as well as any developed nation, have promoted biofuels such as ethanol. The success of the fuel ethanol industry is directly proportional to the profitability of that industry. One method to improve the profitability is by enhancing pretreatment processes and reducing energy inputs. Many studies have shown that ultrasonication has promising potential in improving starch hydrolysis. The goal of this dissertation is to investigate the use of ultrasonics as potential pretreatment to saccharification and fermentation in ethanol production from corn.

In batch ultrasonication to pretreat commodity corn slurry for saccharification, a 30% improvement in glucose release with respect to an unsonicated control group is achievable for a given hydrolysis time. This increase in glucose release from sonicated samples is due to reduction in particle size, better mixing due to streaming effects, and release of additional protein and lipid-bound starch. Ultrasonics reduces particle size of the ground corn by nearly 20-fold, improving enzymatic hydrolysis. The addition of enzyme during low and medium ultrasound treatment does not cause enzyme to denature but rather improved its activity (saccharification). From the energy efficiency calculation, for each Joule of ultrasound energy introduced, up to 2 Joules of additional energy from sugar is hydrolyzed from starch in a given time period.

A further investigation using sugary-2 corn, a mutant corn categorized for its higher level of sucrose and amylose content, has the same results. Sonicated sugary-2 corn is more easily hydrolyzed by the enzyme compared to the sonicated commodity corn (yellow dent corn). This is because of higher saccharification rate. Similar to conventional heating, swelling (absorption of water) is also present during ultrasound treatment. However, ultrasonication of sugary-2 corn has better swelling and gelatinization compared to conventional heating. In the 96-h saccharification, ultrasonication improves the extent of sugar production by 17-21% relative to the unsonicated control group. Similarly, the reaction rates of the sonicated samples are 2-10-fold higher than the reaction rates of the control samples.

Scale-up is considered an important aspect in the novel of this method. Continuous flow ultrasonication using a "donut" horn, used in large-scale waste activated sludge treatment, was studied for potential scale-up. While the ultrasonic batch system yields higher glucose conversion rate results compared to the continuous system, an ultrasonic continuous flow system is more energy efficient based on ultrasonic energy density and saccharification yield. Particle size analysis due to ultrasonication (batch and continuous flow systems) is also characterized in the study. The reduction of particle size due to ultrasonics is proportional to the ultrasonic energy introduced regardless of the type of system used.

In order to justify ultrasonics as a potential pretreatment method, it was compared with the current pretreatment technology; jet cooking. Simultaneous saccharification and fermentation (SSF) experiments were conducted on ultrasonicated samples and jet cooked samples obtained from an ethanol plant. Fermentation products including ethanol, lactic acid and acetic acid were analyzed in the study. Ethanol conversions based on theoretical yield are comparable between ultrasound treated and jet cooked samples. This implies that the physical and chemical effects due to ultrasonics yield similar results as steam in pre-saccharification. Because no bacterial contamination is found, acetic acid production during SSF is produced by the yeast. An economic analysis also indicates that ultrasonic treatment is more cost efficient compared to jet cooking.

Based on these results, the study concluded that ultrasound treatment is a promising, and more efficient alternative to jet cooking and a potential presaccharification and fermentation treatment method in ethanol production from corn.

Future Studies

1. This study is mainly focused on effects of ultrasonic pretreatment on saccharification and fermentation. Thus, it is recommended that effects on downstream processes and co-products (distiller's dry grains with solubles, thin stillage, corn oil and syrup) should be investigated.
2. This dissertation is limited to lab-scale experimental set-up. It is therefore recommended that a pilot-scale system be developed. It should involve higher volumetric flow rates coupled with different pressures and the use of multiple donut ultrasonic horns. This will determine the effects of pressure and flow rates on the ultrasonic efficiency.
3. It has been known that jet cooking denatures enzymes, even the thermo-stable ones. In contrast, it is seen in this study that ultrasound treatment at low and

medium power did not denature the enzymes. Thus it is believed that the use of ultrasound pretreatment will reduce the amount of enzymes needed in ethanol plants. A comprehensive economic analysis is needed to improve the economic analysis presented in this study. This should include savings from the reduction of enzyme use.

4. This study concluded that ultrasonics treatment is a promising pretreatment method in corn ethanol plants. It is suggested that this technique could also work in other starch based feedstocks such as cassava and sorghum. In addition, studies have also shown the use of ultrasonics during fermentation from cellulosic resources have improved the ethanol yield. However, these studies were conducted in batch system. Therefore, a further investigation is proposed involving continuous flow ultrasonication for potential scale-up.

APPENDIX

Experimental Data

Table A.1 Data for Figure 3.3A

Size (µm)	Control	SD$_{20}$	SD$_{40}$	SA$_{20}$	SA$_{40}$
Volume (%)					
2.188	0.00	0.14	0.09	0.00	0.00
2.512	0.09	0.30	0.26	0.14	0.13
2.884	0.17	0.48	0.47	0.26	0.24
3.311	0.25	0.66	0.68	0.42	0.37
3.802	0.33	0.84	0.90	0.59	0.51
4.365	0.41	0.99	1.10	0.79	0.68
5.012	0.48	1.13	1.29	1.01	0.88
5.754	0.55	1.25	1.45	1.25	1.10
6.607	0.61	1.34	1.59	1.50	1.36
7.586	0.67	1.41	1.69	1.74	1.63
8.710	0.73	1.45	1.76	1.97	1.91
10.000	0.80	1.48	1.80	2.16	2.18
11.482	0.86	1.48	1.81	2.31	2.42
13.183	0.94	1.47	1.79	2.39	2.60
15.136	1.01	1.45	1.75	2.41	2.72
17.378	1.10	1.42	1.70	2.36	2.77
19.953	1.18	1.40	1.66	2.25	2.73
22.909	1.27	1.38	1.62	2.10	2.64
26.303	1.36	1.38	1.62	1.94	2.51
30.200	1.45	1.41	1.67	1.80	2.37
34.674	1.54	1.49	1.77	1.71	2.27
39.811	1.63	1.62	1.95	1.70	2.24
45.709	1.74	1.83	2.19	1.77	2.30
52.481	1.87	2.11	2.49	1.94	2.45
60.256	2.01	2.44	2.83	2.19	2.68
69.183	2.17	2.82	3.17	2.50	2.96
79.433	2.34	3.20	3.47	2.83	3.24
91.201	2.50	3.54	3.68	3.15	3.48
104.713	2.64	3.81	3.77	3.41	3.63
120.226	2.76	3.95	3.72	3.59	3.66
138.038	2.84	3.97	3.53	3.66	3.57
158.489	2.91	3.87	3.25	3.65	3.37
181.970	2.97	3.67	2.91	3.55	3.10
208.930	3.06	3.41	2.57	3.40	2.81

239.883	3.21	3.15	2.31	3.22	2.54
275.423	3.44	2.94	2.15	3.06	2.33
316.228	3.76	2.81	2.14	2.92	2.22
363.078	4.15	2.79	2.26	2.84	2.19
416.869	4.58	2.86	2.50	2.79	2.23
478.630	4.99	3.01	2.79	2.77	2.32
549.541	5.29	3.17	3.07	2.74	2.39
630.957	5.42	3.28	3.25	2.68	2.41
724.436	5.30	3.27	3.29	2.55	2.34
831.764	4.94	3.12	3.15	2.35	2.17
954.993	4.29	2.76	2.80	2.03	1.88
1096.478	3.40	2.22	2.25	1.60	1.49
1258.925	0.00	0.00	0.00	0.00	0.00

Table A.2 Data for Figure 3.3B

Size (um)	**Control**	**SD$_{20}$**	**SD$_{40}$**	**SA$_{20}$**	**SA$_{40}$**
Volume (%)					
2.188	0.00	0.14	0.11	0.00	0.00
2.512	0.09	0.36	0.28	0.12	0.12
2.884	0.17	0.62	0.49	0.22	0.21
3.311	0.25	0.88	0.69	0.35	0.33
3.802	0.33	1.13	0.89	0.48	0.44
4.365	0.41	1.35	1.08	0.65	0.60
5.012	0.48	1.54	1.25	0.87	0.79
5.754	0.55	1.69	1.40	1.12	1.04
6.607	0.61	1.79	1.52	1.43	1.34
7.586	0.67	1.85	1.61	1.77	1.69
8.710	0.73	1.87	1.67	2.13	2.09
10.000	0.80	1.86	1.70	2.50	2.51
11.482	0.86	1.81	1.70	2.83	2.93
13.183	0.94	1.77	1.69	3.11	3.30
15.136	1.01	1.72	1.65	3.30	3.60
17.378	1.10	1.69	1.62	3.39	3.80
19.953	1.18	1.70	1.58	3.37	3.89
22.909	1.27	1.74	1.57	3.26	3.86
26.303	1.36	1.85	1.58	3.07	3.73
30.200	1.45	2.01	1.64	2.87	3.55
34.674	1.54	2.23	1.76	2.70	3.35
39.811	1.63	2.51	1.96	2.59	3.18
45.709	1.74	2.83	2.22	2.60	3.09
52.481	1.87	3.17	2.54	2.72	3.08

60.256	2.01	3.49	2.91	2.95	3.15
69.183	2.17	3.76	3.29	3.25	3.27
79.433	2.34	3.94	3.64	3.57	3.39
91.201	2.50	4.00	3.91	3.86	3.46
104.713	2.64	3.94	4.07	4.05	3.44
120.226	2.76	3.74	4.10	4.13	3.30
138.038	2.84	3.44	3.99	4.07	3.06
158.489	2.91	3.07	3.77	3.88	2.75
181.970	2.97	2.69	3.48	3.61	2.40
208.930	3.06	2.33	3.16	3.27	2.06
239.883	3.21	2.05	2.87	2.89	1.79
275.423	3.44	1.88	2.64	2.51	1.58
316.228	3.76	1.82	2.49	2.15	1.47
363.078	4.15	1.87	2.44	1.81	1.43
416.869	4.58	2.01	2.47	1.51	1.45
478.630	4.99	2.20	2.54	1.25	1.49
549.541	5.29	2.38	2.61	1.02	1.53
630.957	5.42	2.51	2.63	0.83	1.53
724.436	5.30	2.52	2.57	0.67	1.47
831.764	4.94	2.41	2.41	0.53	1.36
954.993	4.29	2.14	2.11	0.40	1.17
1096.478	3.40	1.71	1.68	0.32	0.93

Table A.3 Data for Figure 3.3C

Size (um)	**Control**	**CEU 20**	**CEU 40**	**CU 20**	**CU 40**
Volume (%)					
2.188	0.00	0.20	0.22	0.00	0.00
2.512	0.08	0.37	0.42	0.10	0.08
2.884	0.15	0.55	0.64	0.20	0.15
3.311	0.24	0.73	0.85	0.31	0.23
3.802	0.31	0.89	1.03	0.42	0.30
4.365	0.39	1.03	1.18	0.57	0.38
5.012	0.46	1.14	1.30	0.75	0.48
5.754	0.52	1.23	1.38	0.99	0.61
6.607	0.59	1.30	1.45	1.27	0.79
7.586	0.65	1.36	1.48	1.59	1.02
8.710	0.71	1.40	1.50	1.95	1.31
10.000	0.77	1.44	1.51	2.31	1.66
11.482	0.84	1.48	1.53	2.67	2.05
13.183	0.92	1.52	1.55	2.97	2.47
15.136	1.01	1.57	1.60	3.21	2.92
17.378	1.10	1.63	1.69	3.36	3.36

19.953	1.20	1.72	1.82	3.41	3.77
22.909	1.30	1.85	2.01	3.36	4.13
26.303	1.39	2.01	2.27	3.25	4.43
30.200	1.48	2.23	2.59	3.10	4.67
34.674	1.57	2.49	2.97	2.98	4.83
39.811	1.66	2.81	3.39	2.91	4.93
45.709	1.75	3.18	3.83	2.94	4.96
52.481	1.85	3.56	4.25	3.06	4.94
60.256	1.96	3.93	4.60	3.26	4.85
69.183	2.09	4.25	4.83	3.50	4.70
79.433	2.23	4.48	4.92	3.73	4.48
91.201	2.38	4.58	4.84	3.89	4.17
104.713	2.51	4.52	4.59	3.94	3.80
120.226	2.63	4.32	4.19	3.84	3.36
138.038	2.73	3.98	3.69	3.61	2.88
158.489	2.81	3.54	3.15	3.27	2.39
181.970	2.89	3.07	2.62	2.87	1.93
208.930	3.00	2.62	2.15	2.46	1.52
239.883	3.15	2.24	1.80	2.09	1.19
275.423	3.38	1.96	1.55	1.79	0.95
316.228	3.69	1.81	1.44	1.59	0.81
363.078	4.09	1.78	1.42	1.48	0.75
416.869	4.53	1.84	1.47	1.45	0.78
478.630	4.97	1.96	1.55	1.47	0.86
549.541	5.33	2.07	1.63	1.50	0.96
630.957	5.54	2.13	1.65	1.52	1.06
724.436	5.52	2.11	1.61	1.48	1.11
831.764	5.24	1.99	1.50	1.39	1.11
954.993	4.64	1.74	1.31	1.21	1.02
1096.47	3.73	1.39	1.04	0.97	0.84

Table A.4 Data for Figure 3.4

Sample	**Glucose (g/L)**		
Raw corn	**LOW**	**MED**	**HIGH**
Control	56.22	48.39	54.80
SD$_{20}$	58.71	55.27	66.78
SD$_{40}$	67.49	57.41	68.80
SA$_{20}$	63.58	54.80	48.98
SA$_{40}$	73.90	61.56	42.81
Cooked Corn	**LOW**	**MED**	**HIGH**
Control	55.51	54.68	120.07
SD$_{20}$	59.42	63.46	127.31
SD$_{40}$	61.32	70.46	155.91
SA$_{20}$	56.81	63.82	147.37
SA$_{40}$	72.00	71.29	47.20

Table A.5 Data for Figure 3.5

Time (h)	**Control**	**Control (fit)**	**Sonicated**	**Sonicated (fit)**
	Glucose (g/L)			
0	0.00	0.00	0.00	0.00
1	9.65	0.00	18.91	9.26
2	11.08	5.87	19.09	17.93
3	13.82	10.63	25.75	26.05
4	17.49	14.49	32.03	33.64
5	21.11	17.62	42.41	40.76
6	21.70	20.15	44.96	47.41
7	23.54	22.21	51.67	53.65
8	25.85	23.87	58.79	59.48

Table A.6 Data for Figure 3.7

	Low	**Med**	**High**	**Low**	**Med**	**High**
	Glucose (mg/L)			% difference		
Control	32542.735	32542.735	32542.740			
SD$_{20}$	40316.437	40316.437	40316.440	23.89	23.89	23.89
SD$_{40}$	37883.447	40613.144	37883.450	16.41	24.80	16.41
SA$_{20}$	35094.408	41206.560	35094.410	7.84	26.62	7.84
SA$_{40}$	36281.233	41384.580	36281.230	11.49	27.17	11.49

Table A.7 Data for Figures 4.2 & Figure 4.4

Sonication Time	Power	Glucose (mmol)	Glucose (g/L)	Starch Conversion (%)	Energy Efficiency (%)
0		39.30	7.08	10.94	0.0
5	low	46.30	8.34	12.89	-21.9
10	low	47.05	8.48	13.10	-57.8
15	low	48.89	8.81	13.61	-63.9
20	low	48.50	8.74	13.50	-73.8
40	low	211.37	38.08	58.83	159.0
0		39.30	7.08	10.94	0.0
5	med	99.63	17.95	27.73	413.2
10	med	124.76	22.48	34.72	255.7
15	med	165.58	29.83	46.09	263.1
20	med	187.27	33.74	52.12	216.4
40	med	215.77	38.87	60.05	113.2
0		39.30	7.08	10.94	0.0
5	high	89.07	16.05	24.79	196.8
10	high	101.92	18.36	28.37	102.0
15	high	152.17	27.41	42.35	131.2
20	high	205.88	37.09	57.30	169.1
40	high	242.24	43.64	67.42	102.7

Table A.8 Data for Figure 4.3

	Starch Conversion (%)
Control	13.74
Jet Cooked	56.31
Sonicated at low power	39.18
Sonicated at med power	48.74
Sonicated at high power	56.78

Table A.9 Data for Figure 4.7A & B

Summary of Results	Time	Swelling Power for Sugary Corn (g/g)	Swelling Power for Commodity Corn (g/g)
No treatment	0	0	0
U/S 5	5	2.497	1.974
U/S 10	10	2.478	1.929
U/S 15	15	2.888	1.889
U/S 20	20	3.418	2.618
U/S 40	40	5.039	2.372

	Time (min)	Swelling Power (g/g)				
Temp (°C)		25-37	32-34	40-42	50-52	70-72
No treatment	0	0	0	0	0	0
Heat	15	2.638	2.513	2.682	3.223	3.394
Heat	120	2.604	2.482	2.737	3.266	3.670
Heat	240	2.584	2.512	2.896	3.428	3.878

Table A.10 Data for Figure 4.8A

Time (h)	Control	40 sec	20 sec	Control (fit)	40 sec (fit)	20 sec (fit)
	Glucose (g/L)					
0	0.00	0.00	0.00	0.00	0.00	0.00
2	6.56	35.56	6.05	5.33	35.61	7.74
4	14.59	50.49	30.02	10.14	48.97	14.21
6	18.79	60.72	25.12	14.47	53.99	19.61
8	24.32	47.78	26.37	18.38	55.87	24.12
10	24.41	42.69	29.62	21.90	56.58	27.89
12	26.23	45.34	31.15	25.07	56.84	31.04
14	24.67	44.83	26.73	27.92	56.94	33.67
16	26.87	49.78	24.49	30.50	56.98	35.86
18	36.56	46.24	29.68	32.82	56.99	37.70
20	19.16	52.71	47.25	34.91	57.00	39.23
22	44.37	52.91	40.97	36.80	57.00	40.51
24	36.41	52.60	40.63	38.50	57.00	41.58
48	52.45	56.37	45.89	49.55	57.00	46.37
60	52.41	59.28	47.28	51.62	57.00	46.79
72	49.77	51.78	44.77	52.72	57.00	46.93
96	55.23	56.56	46.24	53.63	57.00	46.99

Table A.11 Data for Figure 4.8B

Time (h)	Control	40 sec	20 sec	Control (fit)	40 sec (fit)	20 sec (fit)
	Glucose (g/L)					
0	0.00	0.00	0.00	0.00	0.00	0.00
2	3.10	27.88	4.94	4.53	17.71	5.73
4	7.34	43.19	9.71	8.62	29.92	10.88
6	11.97	45.08	22.21	12.31	38.34	15.52
8	15.36	44.70	17.81	15.63	44.14	19.69
10	19.77	43.31	25.94	18.62	48.13	23.44
12	18.04	42.38	26.11	21.32	50.89	26.81
14	20.71	36.44	21.74	23.75	52.79	29.84
16	27.62	49.53	28.32	25.95	54.10	32.57
18	28.14	41.53	37.11	27.92	55.00	35.03
20	28.78	52.82	38.91	29.71	55.62	37.24
22	34.50	46.66	40.24	31.31	56.05	39.22
24	36.30	52.41	45.53	32.76	56.34	41.01
48	40.76	55.76	50.03	42.19	56.99	52.51
60	42.70	58.54	56.04	43.96	57.00	54.62
72	44.40	52.52	57.28	44.90	57.00	55.74
96	47.51	57.38	58.23	45.68	57.00	56.65

Table A.12 Data for Figure 5.3

Sonication Time	20s	40s
Samples	**Glucose (g/L)**	
Control	14.48	
Low	20.76	22.13
Med	24.18	60.20
High	33.11	61.63

Table A.13 Data for Figure 5.4

Flow rate (gal/min)	Reducing sugar (g/L)	Relative sugar increase (g/L:g/L)
Control	14.53	0
7.44	18.13	1.25
5.35	25.61	1.76
5	22.35	1.54
4.53	22.93	1.58
4.32	28.26	1.94
3.61	27.65	1.9
2.91	30.22	2.08
2.67	26.09	1.8

Table A.14 Data for Figure 5.5

	Relative Net Energy Gain (J/L:J/L)
Batch (Low power for 40s)	16.32
Batch (Med power for 40s)	1.76
Batch (High power for 40s)	2.26
Continuous (5.35 gal/min)	23.50
Continuous (4.32 gal/min)	23.51
Continuous (3.61 gal/min)	18.58

Table A.15 Data for Figure 5.6

Energy Density (kJ/L)	Starch Conversion (%)
4.4	11.3
6.2	16
6.6	13.9
7.3	14.3
7.7	17.6
9.2	17.2
11.4	18.8
12.4	16.3
87.7	12.8
122.3	14.9
160	19.8
170.3	20.4
260.6	37.1
227.4	37.9

Table A.16 Data for Figure 5.7

Size (um)				Volume (%)
	Control	**Continuous**	**CU20-low**	**CU40-low**
1.445	0	0.02	0	0
1.66	0	0.13	0	0
1.905	0	0.2	0	0
2.188	0	0.23	0	0
2.512	0	0.2	0	0
2.884	0	0.15	0	0
3.311	0	0.1	0	0
3.802	0	0.09	0	0
4.365	0	0.14	0	0
5.012	0.17	0.53	0.46	0
5.754	0.33	0.89	0.87	0.05
6.607	0.51	1.34	1.43	0.3
7.586	0.74	1.84	2.08	0.64
8.71	0.98	2.36	2.75	1.17
10	1.22	2.83	3.38	1.87
11.482	1.43	3.18	3.87	2.69
13.183	1.57	3.36	4.16	3.59
15.136	1.64	3.34	4.2	4.47
17.378	1.61	3.11	3.96	5.24
19.953	1.51	2.72	3.5	5.78
22.909	1.35	2.22	2.89	6.03
26.303	1.17	1.71	2.24	5.93
30.2	1	1.26	1.65	5.51
34.674	0.88	0.93	1.21	4.82
39.811	0.84	0.76	0.96	3.98
45.709	0.87	0.75	0.92	3.12
52.481	0.98	0.86	1.05	2.36
60.256	1.15	1.06	1.3	1.78
69.183	1.34	1.3	1.59	1.43
79.433	1.55	1.52	1.86	1.3
91.201	1.73	1.7	2.07	1.34
104.713	1.88	1.83	2.18	1.48
120.226	2	1.91	2.21	1.65
138.038	2.11	1.97	2.17	1.79
158.489	2.26	2.01	2.1	1.86
181.97	2.48	2.17	2.06	1.85
208.93	2.82	2.37	2.09	1.79
239.883	3.31	2.7	2.23	1.71
275.423	3.94	3.13	2.5	1.64

316.228	4.71	3.66	2.87	1.61
363.078	5.51	4.21	3.32	1.67
416.869	6.27	4.74	3.78	1.8
478.63	6.86	5.13	4.17	1.99
549.541	7.17	5.32	4.41	2.21
630.957	7.1	5.24	4.44	2.4
724.436	6.65	4.89	4.24	2.51
831.764	5.77	4.24	3.74	2.51
954.993	4.55	3.34	3	2.38
1096.478	0	0	0	2.09
1258.925	0	0	0	1.67

Size (um)				
	CU20-med	**CU 40-med**	**CU20-high**	**CU 40-high**
5.754	0.08	0	0.05	0
6.607	0.52	0	0.34	0
7.586	0.98	0	0.66	0
8.71	1.63	0	1.18	0.01
10	2.38	0.01	1.85	0.42
11.482	3.16	0.41	2.63	1.06
13.183	3.92	1.07	3.49	2.02
15.136	4.52	2.07	4.31	3.32
17.378	4.89	3.43	5.02	4.83
19.953	4.97	5.01	5.5	6.45
22.909	4.74	6.72	5.7	7.94
26.303	4.24	8.3	5.58	9.13
30.2	3.55	9.55	5.15	9.79
34.674	2.8	10.26	4.49	9.8
39.811	2.1	10.28	3.7	9.14
45.709	1.57	9.58	2.91	7.89
52.481	1.27	8.27	2.24	6.25
60.256	1.2	6.56	1.75	4.49
69.183	1.33	4.7	1.48	2.83
79.433	1.61	2.96	1.43	1.49
91.201	1.95	1.55	1.53	0.54
104.713	2.28	0.54	1.72	0.06
120.226	2.55	0.05	1.91	0
138.038	2.71	0	2.06	0
158.489	2.77	0	2.13	0.1
181.97	2.73	0.05	2.12	0.37
208.93	2.63	0.37	2.03	0.58
239.883	2.5	0.58	1.91	0.72
275.423	2.39	0.73	1.8	0.81

60.26	0.98	0.96	0.82	0.81
69.18	1.15	1.20	1.04	0.88
79.43	1.34	1.48	1.30	1.02
91.20	1.55	1.75	1.55	1.20
104.71	1.73	1.98	1.75	1.38
120.23	1.88	2.15	1.89	1.55
138.04	2.00	2.25	1.97	1.72
158.49	2.11	2.31	2.03	1.89
181.97	2.26	2.35	2.10	2.10
208.93	2.48	2.42	2.23	2.38
239.88	2.82	2.56	2.46	2.76
275.42	3.31	2.78	2.80	3.28
316.23	3.94	3.09	3.25	3.92
363.08	4.71	3.47	3.79	4.65
416.87	5.51	3.87	4.35	5.40
478.63	6.27	4.24	4.85	6.08
549.54	6.86	4.48	5.20	6.58
630.96	7.17	4.54	5.32	6.81
724.44	7.10	4.39	5.16	6.69
831.76	6.65	4.02	4.74	6.22
954.99	5.77	3.42	4.04	5.37
1096.48	4.55	2.66	3.14	4.22

Table A.18 Data for Figure 5.11

	Weighted Mean Particle (µm)	**Energy Density (J/L)**
Control	392.21	0.00
Continuous U/S	300.47	5113.45
CU 20 Low	261.21	86285.71
CU 20 Med	185.11	123714.29
CU 20 High	171.01	169428.57
CU 40 Low	166.24	162857.14
CU 40 Med	82.74	241714.29
CU 40 High	92.47	284571.43

316.228	2.31	0.79	1.74	0.87
363.078	2.3	0.8	1.75	0.9
416.869	2.34	0.76	1.85	1.01
478.63	2.43	0.72	2.02	1.07
549.541	2.54	0.68	2.22	1.12
630.957	2.63	0.64	2.41	1.14
724.436	2.66	0.61	2.53	1.1
831.764	2.6	0.58	2.54	0.99
954.993	2.43	0.53	2.42	0.8
1096.478	2.12	0.45	2.14	0
1258.925	1.69	0.36	1.71	0

Table A.17 Data for Figure 5.8

Particle Size (um)	**Control**	**7.45**	**4.54**	**3.29**
	Volume (%)			
1.45	0.00	0.02	0.02	0.00
1.66	0.00	0.13	0.12	0.00
1.91	0.00	0.22	0.20	0.00
2.19	0.00	0.25	0.22	0.00
2.51	0.00	0.24	0.20	0.00
2.88	0.00	0.18	0.15	0.00
3.31	0.00	0.13	0.09	0.00
3.80	0.00	0.12	0.07	0.00
4.37	0.00	0.17	0.12	0.00
5.01	0.05	0.33	0.26	0.00
5.75	0.17	0.59	0.50	0.04
6.61	0.33	0.97	0.85	0.19
7.59	0.51	1.45	1.29	0.46
8.71	0.74	1.99	1.80	0.76
10.00	0.98	2.54	2.32	1.12
11.48	1.22	3.05	2.80	1.50
13.18	1.43	3.43	3.17	1.84
15.14	1.57	3.63	3.36	2.13
17.38	1.64	3.61	3.35	2.30
19.95	1.61	3.36	3.13	2.34
22.91	1.51	2.94	2.73	2.25
26.30	1.35	2.40	2.22	2.04
30.20	1.17	1.85	1.69	1.75
34.67	1.00	1.36	1.23	1.44
39.81	0.88	1.01	0.89	1.16
45.71	0.84	0.83	0.71	0.95
52.48	0.87	0.82	0.70	0.83

Table A.19 Data for Figure 6.5

	Ethanol Yield (g/L)					
Time (h)	0	6	12	18	24	48
Samples						
Control	0.530	4.338	10.47	15.299	20.505	27.085
Corn Slurry	0.545	4.56	31.621	33.792	34.635	35.382
Jet Cooked	0.499	4.45	33.647	35.454	37.735	37.745
Batch U/S	0.540	5.04	33.596	34.763	37.267	38.72
Continuous U/S	0.464	5.01	28.52	29.76	30.943	32.59
	Glucose (g/L)					
Control	3.88	3.26	0.87	0.86	0.77	0.86
Corn Slurry	39.77	52.44	3.2	1.46	1.36	1.22
Jet Cooked	42.16	57.49	3.16	1.48	1.34	1.31
Batch U/S	44.15	56.53	3.16	2.25	1.78	1.27
Continuous U/S	39.79	45.78	3.17	1.07	0.94	0.82

Table A.20 Data for Figure 6.6A

	Yeast Count (10^6 cells/ml)	
	24th hour	**48th hour**
Control	2.6	2.76
Corn Slurry	2.97	4.7
Jet Cooked	3.06	5.27
Batch U/S	2.06	6.52
Continuous U/S	2.35	4.27

Table A.21 Data for Figure 6.6 B & C

	Lactic Acid (g/L)				
Time (h)	**6**	**12**	**18**	**24**	**48**
Control	0	0	0	0	16.02
Corn Slurry	0	0	0	0	0.91
Jet Cooked	0	0	0	0	1.2
Batch U/S	0	0	0	1.51	0.71
Continuous U/S	0	0	0	1.54	4.9
	Acetic Acid (g/L)				
Control	0.134	0.164	0.26	1.395	5.858
Corn Slurry	0.135	0.224	0.241	0.329	0.893
Jet Cooked	0.139	0.235	0.296	0.367	1.554
Batch U/S	0.15	0.273	0.302	0.405	0.95
Continuous U/S	0.14	0.274	0.357	0.671	4.518

Table A.22 Data for Figure 6.7

	Theoretical Ethanol Conversion (%)					
Time (h)	0	6	12	18	24	48
Samples						
Control	1.09	8.66	20.19	28.44	36.7	44.75
Corn Slurry	1.08	8.72	58.4	60.18	59.39	56.01
Jet Cooked	1.06	9.14	66.7	67.78	69.46	64.14
Batch U/S	1.08	9.64	62.04	61.91	63.91	61.29
Continuous U/S	1.04	10.86	59.72	60.09	60.16	58.49

Table A.23 Data for Figure 6.8

Number of Ultrasonic Units	***Annual Production Cost using Ultrasonics***	***Difference in Annual Production Cost of Jet Cooking and Ultrasonics***
20	$483,161	$223,946
30	$499,699	$207,408
40	$516,238	$190,869
50	$532,776	$174,331
60	$549,314	$157,793
70	$565,852	$141,255
80	$582,390	$124,717
85	$590,660	$116,447
90	$598,929	$108,178
100	$615,467	$91,640
120	$648,543	$58,564
150	$698,158	$8,949
160	$714,696	-$7,589
180	$747,772	-$40,665

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DEDICATION

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The author also wishes to dedicate this dissertation to her husband, Gilson and her family in Philippines: to her mother, Susana; her late father, Mario Sr.; her brothers (Mario Jr., Andrew, Sonny, Rommel and Richard) and their families. This is especially dedicated to her late sister, Emelita. They may not be physically present as she completes her studies but she has always been grateful and proud to have them as her family.

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