Ultrasonic pretreatment of energy cane bagasse for biofuel production

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ULTRASONIC PRETREATMENT OF ENERGY CANE BAGASSE FOR BIOFUEL PRODUCTION

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Biological and Agricultural Engineering

by
Niyaz Ahamed Methrath Liyakathali
B.E, Sri Ramakrishna Engineering College (Anna University), 2008
May 2014
Heart

To my beloved family
ACKNOWLEDGMENTS

I would like to express my deep gratitude to Dr. Dorin Boldor for offering me this research opportunity and believing in me. Your guidance, encouragement and advice throughout the academic years gave me confidence to achieve what I was able to do. I would also like to thank Dr. Steven Hall and Dr. Chandra Theegala for accepting to serve on my committee and for being very concerned about my work.

I would also like to thank the following members in Dr. Boldor’s group for their patience in helping me in the lab and with my thesis work: Pranjali Muley, Laura Picou Fennell, Charles Henkel, and Brianna Bourgeois. Special thanks to Brianna for spending her time in helping me with the thesis report. I would also like to thank the office staff of the Department of Biological & Agricultural Engineering at LSU, Ms. Angela Singleton, Ms. Donna Elisar and Mr. Thomas McClure for being a part of it.

I am grateful to Dr. Giovanna Aita for patiently answering my technical questions and allowing me to work in her lab at Audubon Sugar Institute, Louisiana. I would also like to thank all her lab members: Dr. Swetha Mahalaxmi, Saeed Oladi, Akanksha Kanitkar, and Zenghui Qiu for helping me in my lab experiments. Special thanks to Dr. Chardcie Verret for her assistance to access the HPLC instrument.

Finally, I would like to thank my parents Mr. M.S. Liyakath Ali and Mrs. Fareedha Liyakath Ali and family for their support and encouragement all throughout my life. It would be difficult for me to pursue higher studies in United States without the inspiration from my elder brother Noushad Akbar; thank you for your motivation, moral support and advice at times. I also like to thank all my friends and fellow mates during my stay in LSU.
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ABSTRACT

High demand for energy and increasingly expensive petroleum prices led to development of new alternative fuels for transportation, such as bio-ethanol and bio-diesel. Even though a cost reduction in the production of cellulolytic enzymes is occurring, the conversion of plant cellulose into sugars still remains an expensive and slow process. Pretreatment of lignocellulose materials to remove lignin and alter physical/chemical structures significantly improves hydrolysis of cellulose to give high yield of sugars. In this study, ultrasonic pretreatment of energy cane bagasse was used in the presence of ammonia (NH₄OH) to enhance the saccharification process by separating lignin, cellulose and hemicellulose from each other in biomass.

The process performance was investigated as a function of low ultrasonic frequency (20, 20.5, 21 kHz) at a power level of 100 W for the reaction time of 30 min at 80 to 90°C reaction temperature. The pretreatment was performed for four different combination mixtures: (1) energy cane bagasse with 28% NH₄OH and water at a ratio of 1:0.5:8 (w:w:w) and processed immediately, (2) energy cane with water at a ratio of 1:8.5 (no ammonia) and processed immediately, (3) energy cane soaked with 28% NH₄OH and water with the same ratio for 3 hours, then drained and pretreated ultrasonically and (4) energy cane soaked with water with the same ratio for 3 hours (no ammonia), then drained and pretreated ultrasonically. Composition analyses were performed after pretreatment to quantify glucose yield and lignin removal rates. Enzymatic hydrolysis tests were also performed to quantify the sugar yield. Results for composition analysis for different pretreatment combinations were obtained against the control. The objective was to find the best frequency for which high glucan % and cellulose digestibility % for degrading them to simple sugars were obtained. The pretreatment process was performed again for all the four combination mixtures mentioned as a function of pretreatment reaction time.
(30, 45, 60 min) with constant frequency at 20 kHz, power level of 200 W and increase in reaction temperature of above 120 °C. All the energy cane combination mixtures were pretreated along with their respective controls (without ultrasonic frequency).

The cellulose digestibilities among various combination mixture samples based on a particular frequency were not statistically significant but varied significantly compared to the untreated energy cane bagasse. The energy cane bagasse with 28% NH₄OH and water for 60 min reaction time obtained the highest cellulose digestibility of almost 44% for both non-soaked and soaked samples compared to 20.44% for the untreated energy cane bagasse (control). Energy cane bagasse with water for 60 min reaction time obtained cellulose digestibility of 34.14%, whereas the water soaked and drained sample for 60 min reaction time obtained cellulose digestibility of 38.12%. The maximum theoretical glucose yield was 24.29 g / 100 g of dry biomass for the combination mixture of energy cane bagasse with 28% NH₄OH and water for 60 min reaction time. Theoretical glucose yield for energy cane bagasse with 28% NH₄OH and water soaked and drained sample for 60 min reaction time was 23.99 g / 100 g of dry biomass, whereas the theoretical glucose yield for water (no ammonia) for 60 min reaction time was 10.07 g / 100 g of dry biomass. Theoretical glucose yield for water soaked and drained sample for 60 min reaction time was 10.91 g / 100 g of dry biomass. The results also indicated that pretreatment time and various combination mixtures were statistically significant at the 95% confidence interval for % glucose yield of pretreated energy cane bagasse. The pretreatment efficiency was also observed via increased porosity and fiber swelling of the treated energy cane fibers through Scanning Electron Microscopy (SEM). These results demonstrated that ultrasonic pretreatment along with NH₄OH can be used as a potential pretreatment method for lignocellulosic biomass to produce biofuels.
CHAPTER 1. INTRODUCTION

1.1 Importance of biofuels

Petroleum is the largest source of energy in the United States (40%) and in the world (35%) (Wyman, Dale et al. 2005). Of the total petroleum consumed in the US, two thirds is used in the transportation sector, which is almost totally dependent on it (>96%). Among all industries, this sector is the largest emitter of carbon dioxide, a proven greenhouse gas (Wyman, Dale et al. 2005). As such, there is a critical need for fuel sources for transportation that can be derived from renewable resources in order to reduce the greenhouse effect, which has been blamed for climate variability and global warming (Little 2000).

A potential solution to partially solve the problem of environmental pollution from fossil fuels used in transportation leads to utilization of alternative fuel for transport. Biomass is a viable, abundant, renewable resource that can be converted to three products: (1) electrical energy, (2) transportation fuel (Sun and Cheng 2002) and (3) chemical feedstocks (McKendry 2002). Depending on the conversion technology used, biomass can produce liquid bio-based fuels such as biodiesel, ethanol, methanol, or bio-oil (Hoekman 2009). Whereas some of these can be used directly in various types of engines (i.e. ethanol, methanol, biodiesel), bio-oil needs to be further refined into gasoline, diesel and jet fuel compounds (Huber and Corma 2007). Ethanol has a lower energy density than gasoline, but it also has a lower stoichiometric air-fuel ratio than gasoline, so a higher compression ratio can be achieved in the engine. These factors produce high output power and improved engine efficiency compared to gasoline-fueled vehicles. According to the Renewable Fuels Association (2003), the US annual fuel ethanol capacity was 2.9 x 10^9 US gallons in 2002, an increase of 10^9 US gallons over the production level in 2000 (Mosier, Wyman et al. 2005), showing that there was an expected demand for
ethanol. As per 2012, the US annual fuel ethanol production was 13.3 billion of gallons (Renewable 2013).

1.2 Lignocellulose as a biofuel feedstock

Lignocellulose is the most abundant renewable biomass and its annual production has been estimated in \(1 \times 10^{10}\) MT worldwide (Sánchez and Cardona 2008). The ethanol production process from lignocellulose biomass involves pretreatment, hydrolysis, fermentation and product separation or purification (Mosier, Wyman et al. 2005). Of these operations, pretreatment is among the most important since a major issue in enzymatic bioprocessing is its slow reaction rate (Figure 1.1.). Pretreatment speeds up the reaction rate by breaking down the outer layer of lignin to allow enzyme access to the cellulose and hemicellulose; which can be more easily hydrolyzed into sugars, necessary for ethanol production.

![Figure 1.1. Schematic diagram for pretreatment on lignocellulosic material (Adapted from (Hsu 1997)).](image)

Lignocellulosic biomass consists of cellulose, hemicellulose, and lignin. Pretreatment separates these components from each other and the sugar monomers obtained after enzymatic hydrolysis are biochemically converted (i.e., fermented) into biofuels. Cellulose is a polymer made up of \(\beta\)-D-glucose units linked by 1,4-\(\beta\)-glucosidic bonds (Figure1.2).
Hemicelluloses are made up of five carbon (pentoses) and six carbon (hexoses) sugars. They differ from cellulose because they have shorter chains and side groups. Lignin is the compound that gives strength to the biomass structure. The lignin components trans-coniferyl, trans-sinapyl, and trans-p-coumaryl alcohols shown in Figure 1.3 are interlinked by hydrogen bonds and glycosidic linkages. Chemical treatment is required in order to separate these components (Feldman 1985).

The lignin macromolecule is formed by interlinking of the above three units through ether linkages at α and β positions.

The presence of lignin and hemicellulose inhibits the access of cellulolytic enzymes, thus reducing the efficiency of the hydrolysis (Mansfield et al., 1999). The degradation of lignin
depends on whether the medium is acidic, alkaline, oxidative, or a combination of all (Hon and Shiraishi 2000). Removing of lignin and hemicellulose, and increasing porosity in the pretreatment process, significantly improves hydrolysis (McMillan James 1994). Many of the crops used for food production, such as corn, wheat, barley, sugarcane, rapeseed, soybean, and sunflower (Aita and Kim 2010) are also used for biofuel production either from their primary products or from their residues. The traditional lignocellulosic biomass sources like crop residues, hardwood, softwood, herbaceous biomass, cellulose waste, and municipal solid wastes have high potential for biofuels production (Aita and Kim 2010).

Energy cane is a promising lignocellulose material to be used in biofuel production. It is a hybrid commercial sugar cane, with reduced fertilizer and water requirement compared to sugarcane. It has high fiber and low sucrose content compared to sugarcane (Kim and Day 2011). Energy cane cultivation and harvesting are already developed due to existing sugarcane infrastructure, but it can be produced with lower cost. In Louisiana, it was shown to be more economical to use energy cane for cellulosic ethanol production because of the state’s favorable climate (Brown 2012). Energy cane exhibits superior production characteristics such as low energy inputs, high potential biomass yield and wider geographical distribution compared to sugar cane (Monge, Ribera et al. 2013). Unlike sugarcane, which requires replanting every 3 years in order to maintain high sugar production, energy cane needs replanting only once in every 10 years as the fiber is the most important product (Sierra et al., 2008), and the harvesting cycle known as stubbling lengths is longer for energy cane (Darby and Salassi 2009). Chemical composition of energy cane was reported to be 43% cellulose, 24% hemicellulose, and 22% lignin which was almost similar to the sugarcane (Kim and Day 2011). Energy cane contains 53.6% juice wet basis; of that 9.8% is sugars in which sucrose is 9.6% and glucose is 0.1% (Kim
and Day 2011). Compared to sweet sorghum and sugarcane, energy cane can produce double the amount of ethanol from cellulose (Kim and Day 2011).

1.3 Effect of pretreatment on saccharification

Pretreatment is performed in order to overcome biomass recalcitrance and improve downstream conversion efficiency. The pretreatment includes the reduction of size, redistribution of the components, depolymerization and solubilization. Both physical and chemical effects take place in the pretreatment process. The physical effect increases the surface area and allows enzyme penetration into the cell walls; the chemical effect depolymerizes and breaks the crosslinking between the macromolecules (Alvira, Tomás- Pejó et al. 2010). Pretreatment is the main process in the production of ethanol because it reduces the cost and makes the overall conversion process more efficient; in its absence the ethanol production time increase several folds (McMillan James 1994). However, thirty percent of the total processing cost in the biomass to ethanol conversion is due to the biomass pretreatment because of the equipment and severe treatment conditions used (Alvira, Negro et al. 2010). Pretreatment affects the cost of most other operations including size reduction prior to pretreatment and enzymatic hydrolysis after pretreatment (Wyman, Dale et al. 2005).

Many pretreatment methods exist depending on the biomass feedstock that yields the highest amount of ethanol (Hsu 1997). Depending on their general principle of operation they can be classified as chemical, biological and physical pretreatments (Alvira, Tomás-Pejó et al. 2010). The biological pretreatment takes days to weeks and needs further treatments to remove contaminants and yield high amounts of sugars. A wide range of chemical pretreatment technologies have been developed based on the use of different chemicals including those based on pH modifications. The high pH in the pretreatment removes lignin, solubilizes hemicellulose
and increases the surface area to allow easier access to the enzymes. There are different solvents used in the pretreatment based on needs. Low pH solvents like dilute acids and neutral pH solvents like water are used in the pretreatment for hemicellulose solubilization and lignin relocation. High pH alkaline solvents are used for removal of a high fraction of the lignin and less hemicellulose loss, so high pH solvents are used in order to produce ethanol by removing lignin in order to access the sugars for ethanol fermentation. Ammonia-based pretreatment increases the surface area of cellulose and has shown better delignification results with low toxicity in improved enzyme efficiency and microbial activity compared to other pretreatments (Kim and Lee 2006). It was observed that alkaline pretreatment of sorghum bagasse produced low lignin content; a high lignin content was obtained with acidic pretreatment (Goshadrou, Karimi et al. 2011). Chemical pretreatment methods using ionic liquids (IL) are very effective in the pretreatment of energy cane, but at a very high cost (Qiu, Aita et al. 2012). In addition to the high cost, the problems with the ionic liquid chemical pretreatment include difficulty in recycling ILs and toxicity to enzymes and microbes. Whereas each pretreatment has its own advantages and disadvantages, none of them have emerged yet as a leading technology suitable for commercialization.

In this study, the use of ultrasound as a pretreatment technique was investigated for improving ethanol production from energy cane biomass. Ultrasound was considered as the practical pretreatment option because scanning electron microscopy images demonstrate that ultrasound has the capacity to modify the surface structure of lignocellulosic biomass (Zhang, Fu et al. 2008). It was also showed that ultrasound has a beneficial effect on the saccharification process (Rolz, de Arriola et al. 1986). As such, it is an emerging technology that has potential as an alternative pretreatment method. Since ultrasound technology reduces the reaction time and
the chemical loading, it can be considered as green technique (Bussemaker and Zhang 2013). It was also demonstrated in previous studies that ultrasonic pretreatment has the potential to improve separation and hydrolysis of components in energy cane and other biomass for biofuel production. Both the chemical and physical structure of lignocellulose was affected by sonochemical and mechanoacoustic effects produced by the ultrasound (Bussemaker and Zhang 2013). In the limited data available in literature regarding ultrasonic pretreatment of lignocellulosic biomass, other researchers have observed that saccharification of cellulose was enhanced by ultrasonic pretreatment (Yachmenev, Condon et al. 2009).

The pretreated energy cane bagasse is then enzymatically hydrolyzed to obtain sugars from cellulose and hemicellulose. The full conversion to biofuel includes not only the hydrolysis of cellulose in the lignocellulosic materials to reducing sugars, but also fermentation of the sugars to ethanol. The hydrolysis is catalyzed by cellulase enzymes, and the fermentation is processed by bacteria or yeasts. The enzymatic hydrolysis method is more efficient and proceeds under ambient temperature conditions without generation of any toxic waste. This method also improved recently in terms of cost and efficiency (Mishima, Tateda et al. 2006). A promising pretreatment requires less intense input energy, minimization in the loss of cellulose and hemicellulose, increased sugar yield after enzyme hydrolysis and low capital costs (Eggleston 2010).

1.4 Principle and effect of ultra-sonication

Ultrasound is generated by a transducer made from a piezoelectric material (Ushakov 2005). The piezoelectric material produces characteristic mechanical vibration of ultrasonic frequency in response to an alternating current. Beyond the audible range, that is at frequency higher than 20 kHz, the ultrasonic waves produce pressure differences in the solution medium
that enhances chemical and physical processes (Mason and Lorimer 2003). Ultrasound produces sonochemical and mechanoacoustic effects which affects the chemical and physical structure of lignocellulose (Bussemaker and Zhang 2013). Generally, ultrasound frequency in liquid medium produces two primary effects (Figure 1.4): cavitation and heating (Yachmenev, Condon et al. 2009). Cavitation occurs at low frequency (16 to 100 kHz) in which most of the ultrasound energy is dissipated into the medium whereas heating occurs at high frequency (> 100 kHz); in this case only small amounts of energy are dissipated. High pressure, compression and low pressure, rarefaction are produced by the pressure waves in the liquid medium. The cavitation bubbles contract and expand with the compression and rarefaction in order to bring more molecules into the bubble process (Peregrine 1994). The collapse of the cavitation bubble produces localized temperatures of ~5500°C and a pressure of 500 atm which gets dissipated into the medium and is restricted to a particular place near the surface of substrate in the medium. A higher acceleration results in a higher fraction of the energy transformed to cavitation. Shearing forces are created in the liquid surrounding the cavitation bubble resulting in a strong mechanical effect. These effects significantly increase the mass and heat transfer to the surface of the substrate while activating the catalyst transport to the substrate (Yachmenev, Condon et al. 2009). The effect of cavitation results in higher heterogeneity systems (solid – liquid) compared to the initial homogeneous system. The characteristic frequency of transducers limits many research applications using ultrasonics (Bussemaker and Zhang 2013). The quantity, lifetime and implosion pressure of cavitation bubbles produced are influenced mainly by the power of ultrasound (Gierer 1990). In the case of an ultrasonic transducer, the higher the amplitude, the higher the acceleration intensity produced and subsequently the power.

Ultrasonic processing not only provides energy for the reaction, but it can achieve better mixing and more rapid separation of lignocellulose materials. It is important to pass ultrasonic
waves into the reactor in a uniform way. The common understanding of ultrasonic pretreatment is that the powerful vibrations causing the ultrasonic waves could severely damage the biomass structure which will subsequently increase the yield of enzyme hydrolysis (Mason, Paniwnyk et al. 1996).

![Diagram of ultrasound energy](image)

**Ultrasound Energy**

- **Low Frequencies** (16 to 100 kHz)
  - Cavitation

- **High Frequencies** (>100 kHz)
  - Heating

**Cavitation Bubble**

- Temperature 5500 °C
- Pressure 500 atm
- Jet of liquid 500 m/sec

Figure 1.4. Cavitation bubbles formed as an effect of ultrasound (Reproduced from (Yachmenev, Condon et al. 2009))

An ultrasonic setup consists of an ultrasonic frequency generator, transducer, and reactor. Generally the ultrasonic frequency is generated from the electric current supplied to the generator. The ultrasonic transducer converts the electrical signal into a mechanical signal in the form of pressure waves delivered to the reactor containing the solution to be sonicated. The transducer normally used for this purpose is an ultrasonic bath, a plate transducer, or a horn transducer (Mason, Paniwnyk et al. 1996). The power of the generator varies but the characteristic frequency of the transducer is generally fixed. The operating parameters influence
the cavitation intensity. Power is directly proportional to the amplitude of the ultrasonic wave, thus influencing the occurrence of cavitation. The temperature of the medium is also a significant factor for the pretreatment process. The ultrasonic effects are influenced by the chemical species present in the medium, including aqueous, ionic, and organic solvents. The solvent will affect the properties such as surface tension and viscosity of the solution. A recent review found that the geometry of the reactor connected to the transducer affected ultrasonic flow, power transfer and mass transfer within a sonicated medium (Gogate, Sutkar et al. 2011), with tubular or hexagonal reactors minimizing the energy consumption. Lower ultrasonic frequencies such as 20–40 kHz increase the mass transfer (Khanal, Grewell et al. 2007).

Ultrasonic pretreatments of various lignocellulosic biomasses were able to enhance enzymatic hydrolysis. The ultrasonic pretreatment enhanced enzymatic hydrolysis of sugar cane bagasse with an increase in glucose yield of 21.3% (Yachmenev, Condon et al. 2009). Ultrasonic pretreatment can increase yields of glucose in downstream processing, while also reducing the long pretreatment process time and enhancing accessibility and delignification. Reports include improved acid hydrolysis (Velmurugan and Muthukumar 2011) and enzymatic hydrolysis (Velmurugan and Muthukumar 2012, Velmurugan and Muthukumar 2012) of sugar cane bagasse, resulting from the increased removal of lignin in ultrasonic pretreatment. Lignin gets separated by the splitting of lignin-hemicellulose linkages during the ultrasonic pretreatment. Ultrasonic treatment potentially increased the cleavage of bonds within lignin component and also the bonds between lignin and hemicellulose. Reduction of polysaccharides is also important while considering ultrasonic pretreatment for biofuels and bio refinery applications as it will lead to a loss in yield. The efficiency of the pretreatment should be affected by the particle size of the biomass. However, the effects of biomass particle size, density, and
concentration on the ultrasonic processing were not well documented in literature. Different lignocellulosic biomass responds differently for the same conditions of ultrasonic pretreatment (Bussemaker and Zhang 2013). It has been perceived that smaller particle size results in better separation than a larger particle size. However, grinding into smaller particles is an energy consuming and expensive step when considered on a larger scale. An increase in ultrasonic power leads to increase in the ultrasonic effects on liquefaction (Sasmal, Goud et al. 2012), dissolution times, and hydrolysis yields(Yunus, Salleh et al. 2010). However the optimal power was found to be at an intermediate level of 120 W compared to 50, 80, and 200 W in the pretreatment of sugar cane bagasse (Esfahani and Azin 2012).

Until now, no research had been conducted with the ultrasonic processing of energy cane using ammonium hydroxide catalyst in the pretreatment process. Similarly, no reports were found in the research literature utilizing a Multi-Mode Multifrequency (MMM) based generator transducer system on biomass pretreatment. The present study was performed in order to investigate the effect of using the ultrasonic pretreatment technique with ammonium hydroxide on energy cane using an innovative ultrasonic system with clamp-on transducer that can be more easily scaled up.

1.5 Objective

In order to find out how the ultrasonic process affects the enzymatic hydrolysis of energy cane bagasse with different medium such as water and ammonium hydroxide, optimization of the ultrasonic parameters such as ultrasonic frequency ranges (20 kHz, 20.5 kHz, and 21 kHz), temperature and reaction time for the pretreatment process were performed and the influence of these parameters for improved biofuel production in the downstream processing was determined. The final results were quantified by the composition analysis and saccharification process.
This study is separated into two parts: (1) Investigation of the effect of various ultrasonic frequencies on different ratios of water and ammonia mixtures with energy cane bagasse for a constant power level and reaction time, in order to determine the frequency range for optimal glucan yield; and (2) Investigation of ultrasound effects on different ratios of water and ammonia mixtures with energy cane bagasse for various reaction times at increased temperature with a discussion of the combination mixtures which yielded higher glucan.

Overall, the combination of the pretreatment with ultrasonic and high pH solution was expected to remove the lignin and make the enzymatic hydrolysis faster and more cost-efficient while improving ethanol yield.
CHAPTER 2. EFFECT OF VARIOUS ULTRASONIC FREQUENCIES ON ENERGY CANE BAGASSE MIXTURES WITH WATER AND AMMONIA

2.1 Introduction

Lignocellulose biomass is a renewable bio resource which may be used for the production of transportation fuels. The most abundant polysaccharides of the biomass are the cellulose and hemicellulose, which are covered up by the lignin fibers. These polysaccharides are sources of sugars that can be converted into biofuels. Lignin and hemicellulose make it difficult for the cellulolytic enzymes to access the cellulose, consequently reducing the efficiency of hydrolysis (Mansfield et al., 1999). A pretreatment process is required to release the cellulose blocked by lignin so as to make it accessible for enzyme hydrolysis, where cellulose enzymes break down cellulose into its soluble glucose. Removing the lignin and increasing the porosity of biomass in pretreatment processes also significantly improves the hydrolysis process (McMillan James 1994). Pretreatment is necessary in order to overcome the recalcitrance of lignin and to reduce the overall cost for ethanol production because the cost of pretreatment is a significant factor affecting the cellulosic ethanol selling price. There are a number of different pretreatments each with its advantages and disadvantages. Some examples include chemical pretreatment like ionic liquid pretreatment, which is very effective in the pretreatment of energy cane, however it’s a costly process (Qiu, Aita et al. 2012). Another pretreatment is the biological pretreatment which generally takes a long time period from days to weeks and still requires further pretreatment techniques to remove contamination and yield high sugars.

In the limited literature data available reported for ultrasonic pretreatment of lignocellulose, some investigators have reported that ultrasonic pretreatment efficiently enhances the saccharification of cellulose (Yachmenev, Condon et al. 2009). This pretreatment process aims at reducing the energy consumption in ethanol production, which will reduce the
overall cost. Studies show that ultrasonic processing has been achieved by using ultrasonic baths and horn type transducers with their own typical transducer frequency. In this study a more specialized ultrasonic unit with a clamp-on transducer operating on the MMM principle was used to pretreat the lignocellulosic biomass. Moreover, according to the literature review, no research was conducted on the effect of ammonium hydroxide catalyst along with ultrasonic processing of energy cane bagasse.

Energy cane is a commercially available lignocellulosic biomass that has greater fiber content and lower sugar content than sugar cane. Energy cane requires less fertilizer and water, and it is replanted once every ten years, while sugar cane is replanted once every three years (Sierra et al., 2008). Energy cane also has higher expected yields per acre. Researchers at the Louisiana State University Agricultural Center Sugar Research Station in St. Gabriel, LA showed that yields ranging from 25.5 to 44.2 tons (wet) per acre of plant material can be obtained for this crop. In LA, the agricultural production sector has great potential in the production of energy cane (Benedict, L. F., 2013).

This study evaluated the use of various ultrasonic frequencies on different ratios of water and ammonia mixtures with energy cane bagasse by perceiving the changes in chemical composition and enzymatic digestibility of untreated and treated lignocellulose.

2.2 Materials and Methods

2.2.1 Substrate

The energy cane (variety L79-1002) was harvested at the Sugar Research Station of Louisiana State University Agricultural Center in Baton Rouge, LA. The stalks were crushed to press - extract the juice. The crushed stalks were stored in the freezer at -20°C. Before pretreatment, the energy cane was thawed and boiled thrice with water for 6 hours at just below
100°C in a boiler to remove water soluble sugars. Then, the extracted biomass was sun dried to 7.64% moisture in dry basis (see Figure 2.1) and stored in sealed plastic bags until the pretreatment experiments took place. The drained water from the extraction process was collected for sugar analysis. The overall schematic of the process is shown in Figure 2.2.

2.2.2 Ultrasonic pretreatment

The ultrasonic setup (Figure 2.3) consists of the ultrasonic generator (AMMM generators 400W, MPI Ultrasonics, Switzerland) connected to the clamp-on transducer (Branson 502/932R, 20 kHz, Mastersonic, Switzerland) fixed on a metal reactor tube of 250ml volume. A torque of about 20 kg force was applied using a torque gauge on the ultrasonic converter clamping to the reactor tube.
1:0.5:8 – Ratio of the energy cane bagasse, ammonia and water based on weight

Figure 2.2. Schematic block diagram of the overall process

Figure 2.3. Overall arrangement of ultrasonic set up
The generator was controlled and accessed by the National Instruments (NI-VISA Run-Time Engine 4.6.2), AMMM Labview (Lab View Run-Time Engine 2009 SP1) based software for optimizing parameters including frequency and power. The transducer was driven by the generator in the amplitude-power mode and all other parameters were optimized accordingly. The impedance matching between the ultrasonic generator and transducers is tracked automatically by the condition and starting frequency of the process. Based on the design, the system runs automatically for a long time without maintenance. Compared to the horn transducer, these clamp-on transducers can be scaled up easily and the tracking of load enhances maximum energy transfer to the material.

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

1. Energy cane (EC) bagasse with 28% NH₄OH and water at a ratio of 1:0.5:8 (w:w:w)
2. EC bagasse soaked with 28% NH₄OH and water with the same ratio for 3 hours and drained
3. EC bagasse with water at a ratio of 1:8.5 (no ammonia).

The ratio of the combination mixture was taken based on mass. The particle size of energy cane was less than 3mm. All three pretreatment combinations mentioned were exposed to three different frequencies (21 kHz, 20.5 kHz, and 20 kHz) for ultrasonic reacting time of 30 min with 100 W power. The samples obtained after the pretreatment were drained and dried in an oven at 110 °C for 20 hours. Untreated energy cane was used as control. All pretreatment experiments were run in duplicates. The samples were then stored in sealed plastic freezer bags and stored until composition analysis and enzymatic hydrolysis were performed (Figure 2.4).
2.2.3 Chemical composition of energy cane

Untreated and ultrasonic pretreated samples were analyzed for the composition of glucan (cellulose), xylan (hemicellulose), lignin, extractives, solids and ash in the biomass using National Renewable Energy Laboratory (NREL’s) Laboratory Analytical Procedures (LAPs #42618, 42619, 42620, 42621, and 42622). All the samples were run in duplicates. National Institute of Standards and Technology (NIST) standard for energy cane was analyzed simultaneously with the untreated and pretreated samples to ensure the accuracy of the procedures. The results were analyzed according to the corresponding LAP using high performance liquid chromatography (HPLC) (Agilent 1200 Series). The percentage of glucan loss, xylan loss and lignin removed were calculated using the following equations adapted from (Qiu, Aita et al. 2012).
2.2.4 Enzymatic hydrolysis

The hydrolysis of untreated and pretreated energy cane was performed using two commercially available enzymes, Spezyme CP (cellulases) (Genencor, Danisco US Inc., Rochester, NY) and Novozyme 188 (cellobiases) (Sigma Aldrich, St. Louis, MO). A total weight of 50 g (dry weight) of treated and untreated energy cane samples were prepared for enzymatic hydrolysis using the NREL’s LAP TP-510-43629 procedure. The activity of the enzymes used is significant and was evaluated using NREL’s LAP TP-510-42628. The substrates were hydrolyzed with 30 FPU/g glucan of Spezyme CP and 30 CBU/g glucan of Novozyme 188. Experiments were run in duplicates. Hydrolyzed liquid samples were collected after 24 h, 48 h, and 72 h. After hydrolysis, the percentage of glucose released from the samples were determined and analyzed by HPLC (Agilent 1200 Series) with a BioRad Aminex HPX-87P (P), lead form, 300 mm x 7.8 mm (ID), 9 µm columns and a differential refractive index detector (G1362A Agilent).

2.2.5 SEM analysis

Scanning electron microscopy (SEM) (JEOL JSM-6610LV, JEOL USA, Inc., Peabody, MA) was used to observe the changes in physical structure of the energy cane before and after
ultrasonic pretreatment. The SEM was operated at 10 keV. The samples were sputter coated with platinum before imaging in order to prevent charging on the surface of the specimen.

2.2.6 Analysis of hydrolyzed energy cane

The samples collected after 24, 48 and 72 hours of enzymatic hydrolysis were heated on a hot plate at above 100 °C in order to deactivate the enzymes present. The samples were then filtered and collected in HPLC vials for sugar analysis by HPLC (Agilent 1200 Series). The theoretical percentage of cellulose digestibility was calculated using the formula provided in NREL’s LAP TP-510-43630 as described below:

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

Where, Glucose* is the glucose concentration (g/L), Cellobiose is the cellobiose concentration (g/L), biomass is the initial dry biomass concentration before the enzymatic hydrolysis (g/L), 1.053 is the multiplication factor to convert cellobiose into equivalent glucose, f is the cellulose fraction in dry biomass (g/g) and 1.111 is the factor to convert cellulose into equivalent glucose. Statistical analysis was performed using Analysis of variance (ANOVA) in Tukey-Kramer’s adjustments by using SAS version 9.3 software.

2.3 Results and Discussion

2.3.1 Effect of ultrasound on energy cane bagasse composition

The composition data of the untreated and pretreated energy cane bagasse were analyzed and compared in Table 2.1 based on dry weight. The untreated energy cane bagasse composition analysis was 39% glucan, 19.3% xylan and 25.3% lignin which were comparable to composition analysis of energy cane bagasse reported by Aita et al. (Aita, Salvi et al. 2011). Total lignin removal observed was low after the pretreatment. There was no significant removal of glucan and xylan of the pretreated samples as indicated by the solid recovered percentage of the
pretreated samples. The percentage of the solid loss after the pretreatment was very low as indicated in the Table 2.1. There was no significant variation with frequency changes of the pretreatment on energy cane bagasse composition. These results lead to the conclusion that the low pretreatment processing temperature (80 to 90 °C) may have prevented lignin removal, as lignin dissolves between 140 and 160 °C and ammonia losses its softening capability (Puri and Pearce 1986). However, a glucose yield of 18.15% higher could be obtained for the pretreated sample compared to the untreated control. Similarly, maximum hemicellulose obtained from treated sample increased by 49.53% compared to the control. The % recovered solids is high which means solids are not removed after the pretreatment. Also there is no reduction in the lignin % among the different frequencies and different combination mixtures. The lignin % is reduced for the soaked mixtures compared to the non-soaked mixtures.

The experimental data of the composition analysis presented in Table 2.1 was used for statistical analysis. The glucan % compared for the energy cane with water and ammonia mixtures for different frequencies did not show any statistical variations. Similarly the glucan % compared with different mixture combinations also did not show much statistical variations at $\alpha$ =0.05. The detailed statistical results are presented in APPENDIX A.

2.3.2 Enzymatic hydrolysis effect on pretreated samples

As the composition analysis does not necessarily indicate the results in terms of sugars available for fermentation, an enzymatic hydrolysis of untreated and pretreated energy cane bagasse was performed. The cellulose digestibilities obtained for the pretreated energy cane combination mixtures at an enzyme loading of 30 FPU Spezyme CP and 30 CBU Novozyme 188/g glucan did not indicate much difference compared to untreated energy cane (11.14%, 15.91% and 15.92%) at 24 h, 48 h and 72 h of hydrolysis, respectively. Cellulose digestibilities
Table 2.1. Composition Analysis of treated and untreated energy cane bagasse

<table>
<thead>
<tr>
<th>Pretreatment Conditions</th>
<th>Biomass component** (% dry weight)</th>
<th>Recovered solids***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lignin</td>
<td>Glucan*</td>
</tr>
<tr>
<td>Mixtures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B:A:W</td>
<td>21</td>
<td>30.85 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>32.02 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30.98 ± 0.62</td>
</tr>
<tr>
<td>B:W</td>
<td>21</td>
<td>30.67 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.98 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>32.03 ± 0.68</td>
</tr>
<tr>
<td>B:A:W 3Hrs soaked</td>
<td>21</td>
<td>26.04 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.68 ± 0.91</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>29.68 ± 0.91</td>
</tr>
<tr>
<td>Untreated energy cane</td>
<td></td>
<td>25.3 ± 0.62</td>
</tr>
</tbody>
</table>

B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts
B:W – Biomass : Water in 1:8.5 parts
* Lower case letters indicate statistical (Tukey grouping) differences between frequencies for same combination mixture
* Upper case letters indicate statistical (Tukey grouping) differences for a frequency among different combination mixtures
** Others not included
*** Mass of dry biomass after pretreatment
of all pretreated samples were higher than untreated energy cane bagasse as shown in Figure 2.5, but there was no significant statistical difference among the various pretreated samples processed at 20 kHz (APPENDIX A). This lack of significant differences may have been due to several factors, including the low processing temperature (<100°C), and the lack of washing with water after pre-treatment. In the latter case, the ammonium hydroxide could have been stuck to the lignin material, preventing the enzymes access to the energy cane during hydrolysis.

![Figure 2.5. Percent cellulose digestibility after enzymatic hydrolysis](image)

EC – Energy cane bagasse  
B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts  
B:W – Biomass: Water in 1:8.5 parts  
Lower case letters indicate statistical (Tukey grouping) differences between frequencies for same combination mixture  
Upper case letters indicate statistical (Tukey grouping) differences for a frequency among different combination mixtures  
* Modified procedure data

These results thoroughly suggested that there was no significant removal of lignin from the energy cane after the pretreatment. The enzyme mixture contained mostly of cellulase-degrading enzymes which increased the cellulose digestibility percentage of the energy cane.
Due to lack of significant differences in glucan percentages, the hydrolysis for the samples processed at 20.5 and 21 kHz was performed directly without performing the initial acid hydrolysis test in order to save time and resources (NREL’s LAP TP-510-43629 procedure).

2.3.3 Scanning Electron Microscopy (SEM) analysis

SEM images of untreated and pretreated energy cane at frequencies 20, 20.5, 21 kHz are shown in Fig. 2.6. It does not appear that the untreated energy cane had many pores (Fig. 2.6.a). After pretreatment, numerous pores were observed in the energy cane structure (Fig. 2.6 b, c, d), but these images are not necessarily indicative of the process performance.

![SEM images of untreated and pretreated energy cane at different frequencies](image)

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

Figure 2.6. SEM images of (a) Untreated EC; (b) B:A:W at 20 kHz; (c) B:A:W at 20.5 kHz; (d) B:A:W at 21kHz. All images are x1000 Magnification
2.4 Conclusion

The results did not indicate any difference between different combination mixtures for a particular frequency based on the compositional analysis, cellulose digestibility and SEM analysis, suggesting that the frequency for various pretreated samples has no effect (at least in the frequency range investigated). Even though the cellulose digestibility percentage among different frequencies for a particular combination mixture was not significant, it showed statistical variations (APPENDIX A). There is no ideal frequency range for the ultrasonic pretreatment to produce higher glucose yields; each ultrasonic pretreatment is limited by its optimal frequency. However, parameters such as time, temperature and power level enhance the ultrasonic pretreatment process. SEM pictures indicated that pretreatment had some effect on the biomass structure, forming pores compared to the untreated biomass and the statistical data showed significant increase in the percent yield of glucose for the pretreated samples compared to untreated at $\alpha = 0.05$. 
CHAPTER 3. EFFECT OF ULTRASONIC REACTION TIME ON ENERGY CANE BAGASSE MIXTURES WITH WATER AND AMMONIA IN INCREASED REACTION TEMPERATURE

3.1 Introduction

In addition to the frequency, other pretreatment parameters that affect the performance are power, temperature and time. This study evaluated the use of ultrasound for various reaction times in the pretreatment of energy cane combination mixtures by perceiving the changes in chemical composition and enzymatic digestibility of untreated and treated lignocellulose. In a previous study, an ultrasonic treatment with alkali for short time period (5-10 min) did not achieve significant delignification compared to alkali pretreatment without ultrasound (Sun and Tomkinson 2002). This suggests that reaction time can be a significant factor in the ultrasonic pretreatment for the removal of lignin. The ultrasonic performance is greatly influenced by the power level, since higher power produces greater cavitation which induces the structural change in the biomass (Rehman, Kim et al. 2013). A temperature higher than 100°C is desirable for the reaction; these temperatures can be achieved by inserting the reactor in an oil bath to maintain the required temperature during sonication (Rehman, Umer et al. 2013). In this study, the frequency was fixed, while the operating temperature and power were increased, and the reaction time was varied in order to observe the performance of the ultrasonic process with respect to maximizing the sugar production. However, studies are reported in literature that indicate that there may be a limit to reaction times in ultrasonic processing after which an increase in the glucose released may not be observed (Rehman, Kim et al. 2013).

3.2 Materials and Methods

3.2.1 Substrate

The preparation of the substrate was performed as described in Chapter 2.
3.2.2 Ultrasonic pretreatment

The pretreatment was undertaken using the system as described in Chapter 2 with some modifications. The modifications included placing the transducer and the reactor in a mineral oil bath heating system in order to achieve the required process temperature (see Figure 3.1.). As the transducer is exterior to the reactor, this configuration can be achieved even at larger scales.

An equipment breakdown occurred in the 400 W ultrasonic generator during the study and a new, high end configuration ultrasonic generator with a maximum operating power of 1000 W with an updated AMMM Labview software was used for half of the experiments. The non-soaked combination mixture samples were processed using the initial 400 W generator, but the soaked and drained sample mixtures were processed with 1000 W generator even though a maximum of 200 W was used for both the soaked and non-soaked samples.

Each sample was loaded into a metal reactor tube and was inserted in mineral oil present in a steel tub (Figure 3.1). Prior to inserting of the reactor, the mineral oil was heated to a steady state temperature between 120 and 140 °C by a heater with a temperature controller. The ultrasonic pretreatment began when the sample mixture inside the reactor reached the steady state temperature of about 120 to 140 °C.

The temperatures of mineral oil and sample were measured by k-type thermocouple sensors and were recorded using a Pico data logger (TC-08, Pico Technology Ltd, United Kingdom). The four different pretreatment combination samples investigated are:

1. Energy cane (EC) bagasse with 28% NH₄OH and water at a ratio of 1:0.5:8 (w:w:w)
2. EC bagasse soaked with 28% NH₄OH and water with the same ratio for 3 hours and drained
3. EC bagasse with water at a ratio of 1:8.5 (no ammonia)
4. EC bagasse soaked with water at a ratio of 1:8.5 (no ammonia) for 3 hours and drained
The ratios for the combination samples were taken based on mass. The particle size of energy cane was less than 3mm. Each of the four different combination mixtures mentioned were exposed to an ultrasonic frequency of 20 kHz at 200 W for three different reaction times (30 min, 45 min and 60 min). The samples obtained after the pretreatment were drained, washed and dried in an oven at 110°C for 20 hours. Controls for this experiment included untreated energy cane along with the four combination samples without any ultrasonic frequency applied. All the pretreatments were run in duplicates. The samples were then stored in sealed plastic freezer bags until composition analysis and enzymatic hydrolysis were performed.

3.2.3 Chemical composition of energy cane

Chemical composition of the energy cane was determined using the same procedure described in Chapter 2.
3.2.4 Enzymatic hydrolysis

The enzymatic hydrolysis was performed in the same way as described in Chapter 2, with the modifications of concentrations of the enzymes used. The substrates were hydrolyzed with 60 FPU/g glucan of Spezyme CP and 60 CBU/g glucan of Novozyme 188, as opposed to 30 FPU/g glucan of Spezyme CP and 30 CBU/g glucan of Novozyme 188 as described in Chapter 2.

3.2.5 SEM analysis

The SEM analysis was performed as described in Chapter 2.

3.2.6 Analysis of hydrolyzed energy cane

The analysis of hydrolyzed energy cane was performed as described in Chapter 2.

3.3 Results and Discussion

3.3.1 Effect of ultrasound on energy cane bagasse composition

The process temperature was recorded in real time and the graphs were plotted using the data obtained for different combination mixtures samples at various reaction times (for an example of the temperature evolution see Figure 3.2, other graphs are shown in APPENDIX C). The average reaction temperature ranged between 120 and 140°C for all the combination mixtures.

The chemical composition of the pretreated energy cane combinations are summarized in Table 3.1. Ultrasonic pretreated energy cane combinations at reaction temperature above 120°C, 20 kHz, 200 W and 30, 45, 60 min reaction time, respectively, showed an increase in glucan % compared to pretreated energy cane combinations at reaction temperature range of 80 to 90°C in Table 2.1 as described in chapter 2.
Figure 3.2. Pretreatment processing temperature evolution of the biomass and the oil medium for Biomass:Ammonia:Water combination mixture samples at different pretreatment time mentioned in the chart.
For non-soaked combination mixtures using 400 W generator:

The glucan % obtained for the energy cane with ammonia and water at 30, 45 and 60 min reaction time were 43.77%, 45.44% and 50.93% respectively. There was a 21.5% increase in the glucan obtained for the energy cane with ammonia and water treated for 60 minutes reaction time compared to the untreated energy cane control. A correlation was observed between the reaction time and the glucan % obtained. Higher reaction time resulted in higher glucan %. The % recovered solids were low for the mixture controls treated without ultrasonic (0 kHz) compared to the pretreated mixtures (20 kHz).

There was a statistical significant difference between the glucan % for the mixtures treated with and without ultrasonification in both the energy cane with ammonia and energy cane without ammonia combination mixtures (Table 3.1.). The glucan % for the various reaction times of energy cane with ammonia at 0 kHz and energy cane without ammonia at 20 kHz were similar according to the statistical result. The glucan compositions of all the non-soaked mixtures mentioned in Table 3.1 were compared individually with three different pretreated reaction time (30, 45 and 60 minutes) using Tukey-Kramer’s grouping in analysis of variance (ANOVA). The glucan and lignin composition of all the mixtures at maximum reaction time 60 minutes were also compared (APPENDIX B).

The % lignin removal, % glucan and xylan loss for all the non-soaked combination mixtures are summarized in Table 3.2. The lignin removal was calculated based on the % recovered solids after the pretreatment. The glucan losses were higher for untreated mixture controls (0 kHz) compared to the pretreated mixtures (20 kHz). These values indicate that even if the lignin for the pretreated mixture samples was not removed completely from the substrate after the pretreatment, they were broken down enough to increase enzyme conversion
## Table 3.1 Composition Analysis of non-soaked energy cane mixtures after pretreatment

<table>
<thead>
<tr>
<th>Pretreatment Conditions</th>
<th>Biomass component** (% dry weight)</th>
<th>Recovered solids***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lignin</td>
<td>Glucan*</td>
</tr>
<tr>
<td>Mixtures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B:A:W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.75 ± 0.72</td>
<td>43.77 ± 1.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.04 ± 1.79</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26.48 ± 2.76</td>
<td>45.44 ± 1.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.32 ± 1.71</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26.86 ± 0.79</td>
<td>50.93 ± 2.47&lt;sup&gt;a,A&lt;/sup&gt;</td>
<td>25.75 ± 2.05</td>
</tr>
<tr>
<td>B:A:W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.86 ± 0.26</td>
<td>30.65 ± 0.04</td>
<td>17.76 ± 0.35</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.5 ± 0.64</td>
<td>30.46 ± 0.66</td>
<td>17.81 ± 0.56</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.05 ± 0.28</td>
<td>32.01 ± 0.25&lt;sup&gt;B&lt;/sup&gt;</td>
<td>17.71 ± 0.25</td>
</tr>
<tr>
<td>B:W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.02 ± 1.09</td>
<td>32.66 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.55 ± 1.70</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26.97 ± 2.05</td>
<td>32.77 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.75 ± 0.18</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.45 ± 1.26</td>
<td>34.14 ± 0.89&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>18.15 ± 0.42</td>
</tr>
<tr>
<td>B:W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.85 ± 0.40</td>
<td>23.03 ± 0.07</td>
<td>12.68 ± 0.45</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26.02 ± 0.17</td>
<td>23.94 ± 0.09</td>
<td>12.16 ± 1.13</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28.95 ± 0.56</td>
<td>25.74 ± 0.86&lt;sup&gt;C&lt;/sup&gt;</td>
<td>13 ± 0.14</td>
</tr>
<tr>
<td>Untreated energy cane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.71 ± 0.59</td>
<td>40.48 ± 0.69</td>
<td>21.51 ± 1.93</td>
</tr>
</tbody>
</table>

B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts
B:W – Biomass : Water in 1:8.5 parts
* Lower case letters indicate statistical (Tukey grouping) differences between reaction time for same combination mixture
* Upper case letters indicate statistical (Tukey grouping) differences for a reaction time among different combination mixtures
** Others not included
*** Mass of dry biomass after pretreatment
performance. This might also be due to incomplete removal of acid insoluble lignin, since after 15 minutes of ultra-sonication, there was an increase in the acid insoluble lignin and then the lignin started decreasing gradually (García, González Alriols et al. 2012).

The theoretical ethanol yields were calculated using the following equation:

\[
\text{Final Glucose yield from 100g dry biomass} = (\text{Glucose yield after 72 hours} \times \text{Glucan \%}) + 2.66 \text{ grams.}
\]

Where, 2.66 grams is the total glucose obtained from the initial energy cane before pretreatment.

For Soaked combination mixtures using 1000 W generator:

The glucan \% for the mixture with ammonia at 60 min is 47.65\% which is greater than the 38.12\% of the mixture without ammonia. The glucan \% obtained for the energy cane with water at 30, 45 and 60 min reaction time were 30.81\%, 33.07\% and 33.22\% respectively, which were low compared to 42.90\%, 42.40\% and 40.56\% respectively for the energy cane with ammonia and water at 30, 45 and 60 min respectively (Table 3.3), indicating increased performance in the presence of ammonia. The glucan \% did show a statistical difference between the ultrasonic treated (20 kHz) and untreated (0 kHz) samples. The \% lignin removal, \% glucan and xylan loss for all the soaked combination mixtures are summarized in Table 3.4.

It was observed that the soaked energy cane mixture combinations generally showed increased \% lignin removal compared to the non-soaked energy cane mixture combinations (Table 3.4). However, these values may not be compared with enough confidence as two different generator systems were used for the non-soaked and the soaked samples. The \% glucan loss was higher for the combination mixtures without ammonia compared to combination mixtures with ammonia and also \% glucan loss were more for the untreated (0 kHz) compared to the treated (20 kHz). Statistical variations were significant for \% glucan and lignin loss
# Table 3.2 Glucan and xylan loss, lignin removal and glucose yields for non-soaked samples

<table>
<thead>
<tr>
<th>Mixtures</th>
<th>Reaction time (Minutes) / Frequency (kHz)</th>
<th>% Glucan loss</th>
<th>% Xylan loss</th>
<th>% Lignin removed</th>
<th>% Glucan yield after 72 hours*</th>
<th>Ethanol yield g / 100g dry biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B:A:W</strong></td>
<td>30 / 20</td>
<td>7.99 ± 0.09</td>
<td>7.63 ± 0.57</td>
<td>9.88 ± 0.32</td>
<td>43.77 ± 1.69</td>
<td>0.34 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>45 / 20</td>
<td>1.48 ± 0.14</td>
<td>12.04 ± 0.07</td>
<td>13.89 ± 0.94</td>
<td>45.44 ± 1.27</td>
<td>0.42 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60 / 20</td>
<td>0.32 ± 0.01</td>
<td>11.37 ± 0.63</td>
<td>23.83 ± 0.93</td>
<td>50.93 ± 2.47</td>
<td>0.44 ± 0.00&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30 / 0</td>
<td>38.03 ± 0.15</td>
<td>36.06 ± 0.49</td>
<td>26.06 ± 1.01</td>
<td>30.65 ± 0.04</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>45 / 0</td>
<td>37.48 ± 0.51</td>
<td>36.92 ± 0.54</td>
<td>26.26 ± 0.38</td>
<td>30.46 ± 0.66</td>
<td>0.34 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>60 / 0</td>
<td>33.76 ± 0.31</td>
<td>35.11 ± 0.70</td>
<td>23.94 ± 0.13</td>
<td>32.01 ± 0.25</td>
<td>0.35 ± 0.01&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>B:W</strong></td>
<td>30 / 20</td>
<td>49.56 ± 0.81</td>
<td>51.03 ± 1.61</td>
<td>16.26 ± 0.91</td>
<td>32.66 ± 0.88</td>
<td>0.17 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>45 / 20</td>
<td>29.06 ± 0.86</td>
<td>30.77 ± 0.73</td>
<td>11.62 ± 0.70</td>
<td>32.77 ± 0.33</td>
<td>0.20 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60 / 20</td>
<td>26.83 ± 0.24</td>
<td>32.36 ± 0.73</td>
<td>11.76 ± 0.19</td>
<td>34.14 ± 0.89</td>
<td>0.22 ± 0.01&lt;sup&gt;C,A&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30 / 0</td>
<td>51.22 ± 1.09</td>
<td>52.97 ± 1.38</td>
<td>19.50 ± 0.70</td>
<td>23.03 ± 0.07</td>
<td>0.17 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>45 / 0</td>
<td>48.70 ± 1.15</td>
<td>49.78 ± 1.63</td>
<td>13.78 ± 0.37</td>
<td>23.94 ± 0.09</td>
<td>0.20 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>60 / 0</td>
<td>47.20 ± 1.09</td>
<td>49.17 ± 1.20</td>
<td>2.98 ± 0.10</td>
<td>25.74 ± 0.86</td>
<td>0.20 ± 0.01&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are in dry basis
B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts
B:W – Biomass: Water in 1:8.5 parts
* Lower case letters indicate statistical (Tukey grouping) differences between reaction time for same combination mixture
* Upper case letters indicate statistical (Tukey grouping) differences for a reaction time among different combination mixtures
Table 3.3 Composition Analysis of soaked energy cane mixtures after pretreatment

<table>
<thead>
<tr>
<th>Pretreatment Conditions</th>
<th>Biomass component** (% dry weight)</th>
<th>Recovered solids***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lignin</td>
<td>Glucan*</td>
</tr>
<tr>
<td>B:A:W 3Hrs soaked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>23.10 ± 0.46</td>
<td>42.65 ± 0.53\textsuperscript{b}</td>
</tr>
<tr>
<td>45</td>
<td>20.92 ± 0.07</td>
<td>45.61 ± 0.39\textsuperscript{a}</td>
</tr>
<tr>
<td>60</td>
<td>20.05 ± 0.16</td>
<td>47.65 ± 0.27\textsuperscript{a,A}</td>
</tr>
<tr>
<td>B:A:W 3Hrs soaked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>21.96 ± 0.51</td>
<td>42.9 ± 0.31</td>
</tr>
<tr>
<td>45</td>
<td>22.72 ± 0.28</td>
<td>42.40 ± 0.29</td>
</tr>
<tr>
<td>60</td>
<td>22.30 ± 0.36</td>
<td>40.56 ± 0.44\textsuperscript{b}</td>
</tr>
<tr>
<td>B:W 3Hrs soaked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>26.73 ± 0.55</td>
<td>29.98 ± 0.06</td>
</tr>
<tr>
<td>45</td>
<td>25.83 ± 0.28</td>
<td>35.73 ± 0.28</td>
</tr>
<tr>
<td>60</td>
<td>22.66 ± 0.30</td>
<td>38.12 ± 0.20\textsuperscript{c}</td>
</tr>
<tr>
<td>B:W 3Hrs soaked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>22.44 ± 0.45</td>
<td>30.81 ± 0.19</td>
</tr>
<tr>
<td>45</td>
<td>22.78 ± 0.73</td>
<td>33.07 ± 0.43</td>
</tr>
<tr>
<td>60</td>
<td>23.29 ± 0.62</td>
<td>33.22 ± 0.63\textsuperscript{D}</td>
</tr>
<tr>
<td>Untreated energy cane</td>
<td>25.71 ± 0.59</td>
<td>40.48 ± 0.69</td>
</tr>
</tbody>
</table>

B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts
B:W – Biomass : Water in 1:8.5 parts
* Lower case letters indicate statistical (Tukey grouping) differences between reaction time for same combination mixture
* Upper case letters indicate statistical (Tukey grouping) differences for a reaction time among different combination mixtures
** Others not included
*** Mass of dry biomass after pretreatment
Table 3.4 Glucan and xylan loss, lignin removal and glucose yields for soaked samples

<table>
<thead>
<tr>
<th>Mixtures</th>
<th>Reaction time (Minutes) / Frequency (kHz)</th>
<th>% Glucan loss</th>
<th>% Xylan loss</th>
<th>% Lignin removed</th>
<th>% Glucan</th>
<th>% Glucose yield after 72 hours*</th>
<th>Ethanol yield g / 100g dry biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>B:A:W SOAK</td>
<td>30 / 20</td>
<td>7.43 ± 0.60</td>
<td>5.01 ± 0.51</td>
<td>20.10 ± 0.28</td>
<td>42.65 ± 0.53</td>
<td>0.21 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.89 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>45 / 20</td>
<td>5.69 ± 0.65</td>
<td>8.49 ± 0.39</td>
<td>30.53 ± 0.77</td>
<td>45.61 ± 0.39</td>
<td>0.38 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.10 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>60 / 20</td>
<td>0.81 ± 0.08</td>
<td>6.11 ± 0.94</td>
<td>33.81 ± 0.84</td>
<td>47.65 ± 0.27</td>
<td>0.44 ± 0.01&lt;sup&gt;A&lt;/sup&gt;</td>
<td>23.99 ± 0.52</td>
</tr>
<tr>
<td></td>
<td>30 / 0</td>
<td>14.81 ± 0.32</td>
<td>18.47 ± 0.21</td>
<td>28.29 ± 0.42</td>
<td>42.90 ± 0.31</td>
<td>0.37 ± 0.01</td>
<td>17.96 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>45 / 0</td>
<td>17.33 ± 0.53</td>
<td>30.81 ± 1.25</td>
<td>29.66 ± 0.64</td>
<td>42.40 ± 0.29</td>
<td>0.37 ± 0.01</td>
<td>17.80 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>60 / 0</td>
<td>20.15 ± 0.43</td>
<td>31.66 ± 0.63</td>
<td>28.04 ± 0.55</td>
<td>40.56 ± 0.44</td>
<td>0.38 ± 0.0&lt;sup&gt;B&lt;/sup&gt;</td>
<td>18.24 ± 0.05</td>
</tr>
</tbody>
</table>

| B:W SOAK | 30 / 20 | 32.74 ± 1.25 | 10.53 ± 0.77 | 3.21 ± 1.07 | 29.98 ± 0.06 | 0.20 ± 0.00<sup>a</sup> | 8.71 ± 0.16 |
|           | 45 / 20 | 20.75 ± 0.61 | 22.96 ± 1.39 | 10.26 ± 0.37 | 35.73 ± 0.28 | 0.21 ± 0.00<sup>a</sup> | 9.92 ± 0.01 |
|           | 60 / 20 | 14.20 ± 0.36 | 31.93 ± 0.42 | 18.00 ± 0.49 | 38.12 ± 0.20 | 0.22 ± 0.02<sup>LA</sup> | 10.91 ± 0.54 |
|           | 30 / 0  | 34.62 ± 0.55 | 37.24 ± 0.99 | 22.13 ± 0.70 | 30.81 ± 0.19 | 0.22 ± 0.02 | 9.34 ± 0.71 |
|           | 45 / 0  | 30.00 ± 0.24 | 34.29 ± 0.90 | 19.22 ± 0.95 | 33.07 ± 0.43 | 0.27 ± 0.01 | 11.41 ± 0.43 |
|           | 60 / 0  | 25.65 ± 0.42 | 24.04 ± 0.46 | 17.07 ± 0.54 | 33.22 ± 0.63 | 0.28 ± 0.01<sup>C</sup> | 11.97 ± 0.64 |

All values are in dry basis
B:A:W – Biomass: Ammonium hydroxide: Water in 1:0.5:8 parts
B:W – Biomass : Water in 1:8.5 parts
* Lower case letters indicate statistical (Tukey grouping) differences between reaction time for same combination mixture
* Upper case letters indicate statistical (Tukey grouping) differences for a reaction time among different combination mixtures
between all the combination mixtures and among all the different reaction times for the same combination mixture.

There was a significant weight loss in the pretreated samples described in Chapter 3 compared to the pretreated samples described in Chapter 2. This showed an increase in the lignin % removed (Table 3.2).

3.3.2 Enzymatic hydrolysis effect on pretreated samples

Cellulose digestibility for all the four combination mixtures (non-soaked and soaked) of ultrasonic pretreated energy cane is summarized in Figures 3.3.a,b,c and d. Hemicellulose digestibility was not investigated since ethanol yield from cellulose was of significance for this study. However, hemicellulose digestibilities were lower than cellulose due to the enzyme used which mostly targeted cellulose. Xylanases enzyme needs to be added to obtain higher hemicellulose digestibility.

![Graph showing cellulose digestibility](image)

**Figure 3.3.a** Cellulose digestibility after enzymatic hydrolysis of energy cane with ammonium hydroxide and water and the untreated energy cane.

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Figure 3.3.b Cellulose digestibility after enzymatic hydrolysis of energy cane with water and the untreated energy cane.

Figure 3.3.c Cellulose digestibility after enzymatic hydrolysis of energy cane with ammonium hydroxide and water soaked for 3 hours before pretreatment and the untreated energy cane.
Figure 3.3.d Cellulose digestibility after enzymatic hydrolysis of energy cane with water soaked for 3 hours before pretreatment and the untreated energy cane.

Enzymatic hydrolysis of energy cane with ammonia and water for enzyme concentrations of 60 FPU Spezyme CP and 60 CBU Novozyme 188 showed a maximum of 44.05% cellulose digestibility for 72 h. The untreated energy cane showed 19.04%, 19.37% and 20.44% at 24 h, 48 h and 72 h of hydrolysis. There was a linear increase in the cellulose digestibility based on hydrolysis time (24, 48 and 72 h) for each combination samples. The cellulose digestibility was gradually increasing for different combination mixture samples based on the process reaction time of 30, 45, and 60 min.

A 33.88% cellulose digestibility after 72 h was observed for energy cane with water and ammonia mixture sample for 30 min of pretreatment followed by 43.62% for 45 min and 44.05% for 60 min respectively. Similarly for energy cane with water mixture sample, a 17.93% cellulose digestibility was obtained after 72 h for 30 min of pretreatment followed by 21.01% for
45 minutes and 22.31% for 60 minutes respectively. For energy cane with water and ammonia mixture soaked sample, a 21.88% cellulose digestibility was obtained after 72 h for 30 min of pretreatment followed by 38.95% for 45 min followed by 43.73% for 60 min. For energy cane with water mixture soaked sample, a 20.51% cellulose digestibility was obtained after 72 h for 30 min of pretreatment followed by 20.51% for 45 min followed by 20.53% for 60 min. The longer the duration of ultrasound, the higher the amount of % cellulose digestibility, because of more microjetting and microstreaming mechanisms (Esfahani and Azin 2012). The cellulose digestibilities of the untreated controls of the respective mixture combination samples were low compared to the pretreated except for the energy cane with water soaked sample (Table 3.2). The cellulose digestibility of the soaked sample mixture controls were generally high compared to the non-soaked sample mixture controls.

Therefore, the results for ultrasonic pretreated samples varies with different combinations and pretreatment time, and by increasing pretreatment temperature improved the efficiency of pretreatment and enzymatic digestibility of pretreated samples.

In this case, the pretreatment temperature of all the samples were between 120 to 140 °C and a maximum pretreatment time of 60 min resulted in significant improvements of the pretreatment efficiency of pretreated energy cane based on sample combinations. As per the enzymatic hydrolysis data, energy cane with ammonia and water, both soaked and non-soaked samples, yielded a maximum of cellulose digestibility on different generator systems (Table 3.2) and fall under the same category based on ANOVA test. However, further research is still required by varying the process parameters to determine the optimal pretreatment conditions for ultrasonic pretreatment on energy cane to obtain high glucose yield.
The % cellulose digestibility (after 72 h) for the pretreated energy cane with water and ammonia for different reaction time showed significant variations from the results obtained, the highest reaction time of 60 min had the highest rating A in the Tukey-Kramer’s grouping compared to 45 and 30 min. The detailed results of the statistical analysis are presented in APPENDIX B.

3.3.3 Mass balance of sugars before pretreatment

According to the mass balance flow chart in figure 3.4., the mass of initial energy cane \((M_{EC})\) before water extraction in the boiler was 13.60 kg with 41\% (dry basis) of moisture content. Water with an amount of 98.42 kg was added for boiling. After six hours of boiling, the mass of the liquid extracted from the first trial was 90.84 kg; this liquid was evaporated in a boiler to obtain thick syrup liquid. The mass of the syrup liquid was 2.58 kg. Using the refractometer, the percentage of assumed sugar present in the thick syrupy liquid was 7.02\%, by which the mass of the Sugar1 after the first trial was 0.1815 kg. Similarly, the second and third trials were repeated and the mass of Sugar2 and Sugar3 were 0.0100 kg and 0.0175 kg respectively. Therefore, the mass of total assumed sugars from all the three trials after extraction and evaporation, added up to 0.209 kg. Based on these data, the mass of solids (cellulose + hemicellulose) present in the initial energy cane was calculated to be 7.85 kg. The mass of simple sugars in soluble form in initial energy cane was 2.66\% of the total energy cane which is 2.66 g of soluble sugars in 100 g dry biomass. Therefore this amount should be added to the total sugars that can be converted to ethanol of each pretreatment mixture giving total sugars yielded per 100 g dry biomass. The 2.66 g of sugar per 100 g dry biomass was accounted for when calculating the theoretical ethanol yields presented in Tables 3.2 and 3.4.
Figure 3.4. Mass balance flow chart of sugars from energy cane bagasse before and after the boiler extraction process.
Based on the fact that soaked sample mixtures utilized less water, it is expected that the overall energy consumption for the pretreatment would be reduced in this case as the water increases a significant energy penalty if it needs to be heated with the biomass during pretreatment.

Water drained from the sample mixture after 3 hours = grams of water saved

Therefore, energy saved compared to non-soaked samples for heating $Q = M C_p \Delta T$

$C_p = 4180 \text{ J/kg K}$

$\Delta T = $ Difference in temperature = Final – Initial = 138.69 – 25 = 113.69°C = 113.69 K

$M = $ Mass of water drained in Kg

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

$Q = 28.51 \text{ kJ of energy not spent}$

Therefore, 1.73 KJ / g of dry biomass were saved. The best ethanol yield combination (B:A:W soaked) produced 886.34 KJ of energy per 100 g dry biomass. The energy produced for each pretreated combination mixtures of non-soaked and soaked samples are summarized in Table 3.5. The optimum sonication power of ultrasound should be in range of 2-10 W/cm$^3$ (Yachmenev, Condon et al. 2009), whereas it was 0.8 W/cm$^3$ in this research process which is considered as one of the reason for not obtaining much higher yield. Higher sonication power also yields more glucan, since the formation of the bubbles at the tip of the transducer are more which transfer more energy into the medium (Gogate, Sutkar et al. 2011).

The energy and cost of ultrasonic pretreatment in a larger scale needs to be investigated in future. It was reviewed that the energy required for ultrasound pretreatment was $7.2 \times 10^4 \text{ J/g}$ compared to steam explosion and autoclave which were $9.9 \times 10^4 \text{ J/g}$ and $23.3 \times 10^4 \text{ J/g}$ respectively (Velmurugan and Muthukumar 2012). One of the reviews stated that ultrasonic
pretreatment is practicable at laboratory scale but is deficient in large scale as for advanced oxidative processes due to the issue of economic feasibility (Mahamuni and Adewuyi 2010).

Table 3.5. Theoretical ethanol yield and energy produced for non-soaked and soaked pretreated sample mixtures

<table>
<thead>
<tr>
<th>Mixtures</th>
<th>Reaction time (Minutes) / Process reaction frequency (kHz)</th>
<th>NON-SOAKED SAMPLES</th>
<th>SOAKED SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Process reaction energy input (kJ)</td>
<td>Ethanol from 100g dry biomass (kJ)</td>
<td>Ethanol from 100g dry biomass (kJ)</td>
</tr>
<tr>
<td>B:A:W</td>
<td>30 / 20 756.00</td>
<td>16.94 ± 0.96</td>
<td>503.11</td>
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<td></td>
<td>45 / 20 1044.00</td>
<td>22.34 ± 0.57</td>
<td>663.49</td>
</tr>
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<td></td>
<td>60 / 20 1368.00</td>
<td>24.29 ± 0.09</td>
<td>721.41</td>
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<tr>
<td></td>
<td>30 / 0 396.00</td>
<td>11.82 ± 0.64</td>
<td>351.05</td>
</tr>
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<td></td>
<td>45 / 0 504.00</td>
<td>13.44 ± 0.15</td>
<td>399.16</td>
</tr>
<tr>
<td></td>
<td>60 / 0 648.00</td>
<td>13.78 ± 0.39</td>
<td>409.26</td>
</tr>
<tr>
<td>B:W</td>
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<tr>
<td></td>
<td>60 / 20 1368.00</td>
<td>10.07 ± 0.38</td>
<td>299.07</td>
</tr>
<tr>
<td></td>
<td>30 / 0 396.00</td>
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<td>45 / 0 504.00</td>
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<td></td>
<td>60 / 0 648.00</td>
<td>7.69 ± 0.24</td>
<td>228.39</td>
</tr>
</tbody>
</table>

3.3.4 Scanning Electron Microscopy (SEM) analysis

SEM images of pretreated energy cane for different mixtures at 60 minutes are shown in Figure 3.5. Numerous pores were observed in the pretreated energy cane structure compared to their respective controls (no frequency) with different reaction times. The energy cane with water and ammonia and the energy cane soaked with water and ammonia (Figures 3.5 (a,c) and 3.6 (a,c)) seemed to have more pores compared to the energy cane with water and energy cane
soaked with water samples (Figures 3.5 (b,d) and 3.6 (b,d)), but swelling was observed in all the pretreated samples.

Figure 3.5. SEM Images of (a) B:A:W, 20 kHz, 60 min (b) B:W, 20 kHz, 60 min (c) B:A:W soak, 20 kHz, 60 min (d) B:W soak, 20 kHz, 60 min.
Figure 3.6. SEM Images of (a) B:A:W, 0 kHz, 60 min (b) B:W, 0 kHz, 60 min (c) B:A:W soak, 0 kHz, 60 min (d) B:W soak, 0 kHz, 60 min at 1000X Magnification.
3.4 Conclusion

Maximum of about 24% of lignin was removed for energy cane bagasse with NH$_4$OH and water non-soaked sample, and about 34% for energy cane bagasse with NH$_4$OH and water soaked sample for 60 min reaction time, which exhibited significant % cellulose digestibility of almost 44% for both the samples compared to the untreated in terms of cellulose yields. However, different generator systems were used for non-soaked and soaked sample mixtures. A maximum theoretical energy of 886.34 kJ per 100 gram dry biomass was produced from theoretical ethanol yield of 23.99 g per 100 gram dry biomass by the energy cane bagasse with NH$_4$OH and water soaked sample for 60 min reaction time. High ethanol yielded soaked sample saved potential amount of energy compared to non-soaked sample. SEM images of pretreated samples revealed some differences compared to the untreated energy cane bagasse. Statistical analysis performed exhibited significant differences among the combination mixtures and reaction time for percent of glucose yielded.

Potential applications of ultrasound with alkaline pretreatment need to be studied further with various factors and more processing parameter combinations. This study concentrated on the optimization of ultrasonic parameters of clamp-on type transducers along with suitable operating conditions for better glucose yield. Based on the results it was concluded that ultrasonic pretreatment with increased time duration accelerates the enzymatic hydrolysis for increased glucan digestibility. It was also concluded that a further increase in the process temperature may have further increased the glucose yield.
CHAPTER 4. SUMMARY AND FUTURE WORK

Lignocellulosic biomass such as energy cane can be a potentially significant renewable feedstock for production of biofuels. The major issue is the recalcitrant nature of the biomass matrix which should be deconstructed by a cost efficient and less time consuming pretreatment process. This study evaluated the use of clamp-on ultrasonic transducer in a MMM configuration in the presence of ammonia for the pretreatment of energy cane bagasse.

While ultrasonic frequency was not shown to have a significant effect, reaction time and specific ammonia mixtures combinations showed significant differences. All the combination mixtures were tested and compared with their respective controls and untreated energy cane bagasse. Among the different combination mixtures, the energy cane bagasse with water and ammonia treated at 200W, 20 kHz, 120 to 140 °C for 60 min showed a high % glucose yield of 44.05% which could produce 24.35 grams of ethanol from 100 gram dry energy cane bagasse. The SEM images revealed more pores for the pretreated samples compared to untreated and the controls even though the lignin removal was low. It appears that the energy cane bagasse with ammonia and water mixture samples was more effective in the ultrasonic processing than when sample mixtures was soaked for 3 hours and drained. But the reduced yields are probably compensated by the reduction in energy due to not needing to heat and process the drained water. Furthermore increase in reaction temperature would have produced expected results.

Fermentation experiments using the NREL procedure need to be performed in the future, in order to confirm these results, as all the experiments described ended with the enzymatic hydrolysis step, and the results were analyzed based on cellulose digestibility.

The key parameters of the ultrasonic pretreatment should be verified and tested before implementing the technology at a larger scale. Particle size, biomass loading, treatment time and
reactor configuration are important operating parameters that must be considered. Once these settings are finalized, the treatment can be taken to pilot scale challenges and full scale costs of ultrasonic processing can be considered.

The research work in this study was mainly experimental to prove that ultrasonic pretreatment along with alkaline solution can improve efficiency for energy cane bagasse to produce more ethanol. Further detailed research has to be performed with the clamp-on ultrasonic transducer in future considering the optimal processing parameters and condition.
REFERENCES


Brown, K. (2012). The Economic Feasibility of Utilizing Energy Cane in the Cellulosic Production of Ethanol, Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science in The Department of Agricultural Economics and Agribusiness by Kayla Brown BS, Louisiana State University.


APPENDIX A
STATISTICAL ANALYSIS FOR CHAPTER 2

Anova comparison of glucan composition of pretreated energy cane bagasse mixture with water and ammonia for various frequencies.

<table>
<thead>
<tr>
<th>The SAS System</th>
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<tbody>
<tr>
<td>The MEANS Procedure</td>
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**Analysis Variable : GLUCAN**

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| FREQ=20.5 |

**Analysis Variable : GLUCAN**

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<td><strong>Class Levels</strong></td>
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| **Number of Observations Read** | 6 |
| **Number of Observations Used** | 6 |
The GLM Procedure

Dependent Variable: GLUCAN

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</table>

R-Square   Coeff Var Root MSE GLUCAN Mean
0.301050   1.709462   0.728858   42.63667

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREQ</td>
<td>2</td>
<td>0.68643333</td>
<td>0.34321667</td>
<td>0.65</td>
<td>0.5843</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREQ</td>
<td>2</td>
<td>0.68643333</td>
<td>0.34321667</td>
<td>0.65</td>
<td>0.5843</td>
</tr>
</tbody>
</table>

Distribution of GLUCAN

FREQ

GLUCAN

F 0.65
Prob > F 0.5843
The GLM Procedure

Levene's Test for Homogeneity of GLUCAN Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREQ</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Error</td>
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<td>0</td>
<td>.</td>
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<td></td>
</tr>
</tbody>
</table>

The GLM Procedure

Distribution of GLUCAN

![Distribution of GLUCAN](Image)
The GLM Procedure

Duncan's Multiple Range Test for GLUCAN

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.531233</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Means</td>
<td>2</td>
</tr>
<tr>
<td>Critical Range</td>
<td>2.320 2.327</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>FREQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>43.1150</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>A</td>
<td>42.4000</td>
<td>2</td>
<td>20.5</td>
</tr>
<tr>
<td>A</td>
<td>42.3950</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for GLUCAN

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.531233</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>3.0457</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.
Anova comparison of glucan composition of pretreated energy cane bagasse mixture with water (no ammonia) for various frequencies.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>FREQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>43.1150</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>A</td>
<td>42.4000</td>
<td>2</td>
<td>20.5</td>
</tr>
<tr>
<td>A</td>
<td>42.3950</td>
<td>2</td>
<td>20</td>
</tr>
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</table>

The SAS System

The MEANS Procedure
FREQ=20

Analysis Variable : GLUCAN

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>46.0850000</td>
<td>0.1060660</td>
<td>0.2301530</td>
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</tr>
</tbody>
</table>

FREQ=20.5

Analysis Variable : GLUCAN

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.0400000</td>
<td>0.1979899</td>
<td>0.4495683</td>
<td>0.0392000</td>
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FREQ=21

Analysis Variable : GLUCAN

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<tr>
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<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>43.7200000</td>
<td>0.6363961</td>
<td>1.4556178</td>
<td>0.4050000</td>
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</tbody>
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### Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
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</thead>
<tbody>
<tr>
<td>FREQ</td>
<td>3</td>
<td>20 20.5 21</td>
</tr>
</tbody>
</table>

| Number of Observations Read | 6 |
| Number of Observations Used | 6 |

### The GLM Procedure

**Dependent Variable: GLUCAN**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>6.58510000</td>
<td>3.29255000</td>
<td>21.69</td>
<td>0.0165</td>
</tr>
<tr>
<td>Error</td>
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<td>0.45545000</td>
<td>0.15181667</td>
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<td>Corrected Total</td>
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<table>
<thead>
<tr>
<th>R-Square</th>
<th>Coeff Var</th>
<th>Root MSE</th>
<th>GLUCAN Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.935310</td>
<td>0.873331</td>
<td>0.389637</td>
<td>44.61500</td>
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</table>

**Source:** FREQ

<table>
<thead>
<tr>
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<th>F Value</th>
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<tbody>
<tr>
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</tbody>
</table>

<table>
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<td>FREQ</td>
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<td>6.58510000</td>
<td>3.29255000</td>
<td>21.69</td>
<td>0.0165</td>
</tr>
</tbody>
</table>
The GLM Procedure

Levene's Test for Homogeneity of GLUCAN Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREQ</td>
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<td>0</td>
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<td></td>
<td>.</td>
</tr>
<tr>
<td>Error</td>
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<td>0</td>
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</tbody>
</table>
The GLM Procedure

Duncan's Multiple Range Test for GLUCAN

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.151817</td>
</tr>
<tr>
<td>Number of Means</td>
<td>2 3</td>
</tr>
<tr>
<td>Critical Range</td>
<td>1.240 1.244</td>
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</tbody>
</table>

Means with the same letter are not significantly different.
### The GLM Procedure

Tukey's Studentized Range (HSD) Test for GLUCAN

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.151817</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>1.6282</td>
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</tbody>
</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>FREQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>46.0850</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>44.0400</td>
<td>2</td>
<td>20.5</td>
</tr>
<tr>
<td>B</td>
<td>43.7200</td>
<td>2</td>
<td>21</td>
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</tbody>
</table>

The Duncan Grouping Mean N FREQ:

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>FREQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>46.0850</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>44.0400</td>
<td>2</td>
<td>20.5</td>
</tr>
<tr>
<td>B</td>
<td>43.7200</td>
<td>2</td>
<td>21</td>
</tr>
</tbody>
</table>
Anova comparison of glucan composition of pretreated energy cane bagasse mixture with water and ammonia soaked for various frequencies.

### The SAS System

#### The MEANS Procedure

<table>
<thead>
<tr>
<th>FREQ</th>
<th>Analysis Variable : GLUCAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>44.4500000</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>FREQ</th>
<th>Analysis Variable : GLUCAN</th>
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</thead>
<tbody>
<tr>
<td>20.5</td>
<td>Mean</td>
</tr>
<tr>
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<td>44.8300000</td>
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<table>
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<tr>
<th>FREQ</th>
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<tbody>
<tr>
<td>21</td>
<td>Mean</td>
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<tr>
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<td>43.2650000</td>
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</table>

#### The GLM Procedure

**Class Level Information**

<table>
<thead>
<tr>
<th>Class Levels Values</th>
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<tbody>
<tr>
<td>FREQ 3 20 20.5 21</td>
</tr>
</tbody>
</table>

Number of Observations Read 6
Number of Observations Used 6
The GLM Procedure

Dependent Variable: GLUCAN

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
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<th>Pr &gt; F</th>
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<tbody>
<tr>
<td>Model</td>
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<td>1.00730000</td>
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<tr>
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<td>5.68180000</td>
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<td></td>
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</tbody>
</table>

R-Square    Coeff Var    Root MSE    GLUCAN Mean
0.468144    2.271714    1.003643    44.18000

<table>
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<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
<tr>
<td>FREQ</td>
<td>2</td>
<td>2.65990000</td>
<td>1.32995000</td>
<td>1.32</td>
<td>0.3879</td>
</tr>
</tbody>
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<table>
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<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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</thead>
<tbody>
<tr>
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<td>2</td>
<td>2.65990000</td>
<td>1.32995000</td>
<td>1.32</td>
<td>0.3879</td>
</tr>
</tbody>
</table>

Distribution of GLUCAN

F 1.32
Prob > F 0.3879

64
The GLM Procedure

Levene's Test for Homogeneity of GLUCAN Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREQ</td>
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<td>0</td>
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</tr>
<tr>
<td>Error</td>
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</table>

The GLM Procedure

Distribution of GLUCAN

![Distribution of GLUCAN](image-url)
The GLM Procedure

Duncan's Multiple Range Test for GLUCAN

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>1.0073</td>
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</table>

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Means</td>
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<tr>
<td>Critical Range</td>
<td>3.194</td>
</tr>
<tr>
<td></td>
<td>3.205</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.

Duncan Grouping | Mean | N  | FREQ |
----------------|------|----|------|
A               | 44.830| 2  | 20.5 |
A               | 44.445| 2  | 20   |
A               | 43.265| 2  | 21   |

The GLM Procedure

Tukey's Studentized Range (HSD) Test for GLUCAN

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>1.0073</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>4.1939</td>
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</table>

Means with the same letter are not significantly different.
Anova comparison of glucan composition of 20 kHz for different combination mixtures.

The SAS System

The MEANS Procedure
MixtureID=BAW

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID=BAWSOAK</td>
<td>44.445</td>
<td>1.110157</td>
<td>2.4978235</td>
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<tr>
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</tr>
<tr>
<td>MixtureID=BW</td>
<td>46.085</td>
<td>0.106066</td>
<td>0.2301530</td>
<td>0.0112500</td>
</tr>
</tbody>
</table>
The GLM Procedure

Class Level Information
Class     Levels  Values
MixtureID  3       BAW BAWSOAK BW

Number of Observations Read  6
Number of Observations Used   6

The GLM Procedure

Dependent Variable: glucan

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>13.67213333</td>
<td>6.83606667</td>
<td>7.58</td>
<td>0.0671</td>
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<tr>
<td>Error</td>
<td>3</td>
<td>2.70575000</td>
<td>0.90191667</td>
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<tr>
<td>Corrected Total</td>
<td>5</td>
<td>16.37788333</td>
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</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  glucan Mean
0.834792   2.143373   0.949693   44.30833

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
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<td>0.0671</td>
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<td>7.58</td>
<td>0.0671</td>
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</tbody>
</table>
### Levene's Test for Homogeneity of glucan Variance

**ANOVA of Squared Deviations from Group Means**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
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<th>F Value</th>
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<tr>
<td>MixtureID</td>
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<td>0</td>
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<td>.</td>
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<tr>
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<td>.</td>
</tr>
</tbody>
</table>

**Distribution of glucan**

![Distribution of glucan](image)

- **F**: 7.58
- **Prob > F**: 0.0671
The GLM Procedure

Duncan's Multiple Range Test for glucan

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.901917</td>
</tr>
<tr>
<td>Number of Means</td>
<td>2</td>
</tr>
<tr>
<td>Critical Range</td>
<td>3.022</td>
</tr>
<tr>
<td></td>
<td>3.032</td>
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</tbody>
</table>
Means with the same letter are not significantly different.

### Duncan Grouping

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>46.0850</td>
<td>2</td>
<td>BW</td>
</tr>
<tr>
<td>A</td>
<td>44.4450</td>
<td>2</td>
<td>BAWSOAK</td>
</tr>
<tr>
<td>B</td>
<td>42.3950</td>
<td>2</td>
<td>BAW</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.901917</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>3.9685</td>
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</table>

Means with the same letter are not significantly different.

### Tukey Grouping

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>46.0850</td>
<td>2</td>
<td>BW</td>
</tr>
<tr>
<td>A</td>
<td>44.4450</td>
<td>2</td>
<td>BAWSOAK</td>
</tr>
<tr>
<td>A</td>
<td>42.3950</td>
<td>2</td>
<td>BAW</td>
</tr>
</tbody>
</table>
Anova comparison of glucan composition of 20.5 khz for different combination mixtures.

The SAS System

The MEANS Procedure
MixtureID=BAW

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>42.4000000</td>
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</tbody>
</table>

MixtureID=BAWSOAK

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>44.8300000</td>
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</tbody>
</table>

MixtureID=BW

<table>
<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>44.0400000</td>
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</tbody>
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The GLM Procedure

Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID</td>
<td>3</td>
<td>BAW BAWSOAK BW</td>
</tr>
</tbody>
</table>

Number of Observations Read  6
Number of Observations Used  6
The GLM Procedure

Dependent Variable: glucan

<table>
<thead>
<tr>
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<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>6.14573333</td>
<td>3.07286667</td>
<td>8.48</td>
<td>0.0582</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>1.08660000</td>
<td>0.36220000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5</td>
<td>7.23233333</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  glucan Mean
0.849758   1.375403   0.601831   43.75667

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID</td>
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<td>6.14573333</td>
<td>3.07286667</td>
<td>8.48</td>
<td>0.0582</td>
</tr>
</tbody>
</table>

<table>
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<th>Mean Square</th>
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<th>Pr &gt; F</th>
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<tbody>
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<td>6.14573333</td>
<td>3.07286667</td>
<td>8.48</td>
<td>0.0582</td>
</tr>
</tbody>
</table>

Distribution of glucan

F 8.48
Prob > F 0.0582
### Levene's Test for Homogeneity of Variance

#### ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID</td>
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<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The GLM Procedure

Duncan's Multiple Range Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.3622</td>
</tr>
<tr>
<td>Number of Means</td>
<td>2</td>
</tr>
<tr>
<td>Critical Range</td>
<td>1.915 1.922</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>44.8300</td>
<td>2</td>
<td>BAWSOAK</td>
</tr>
<tr>
<td>A</td>
<td>44.0400</td>
<td>2</td>
<td>BW</td>
</tr>
<tr>
<td>B</td>
<td>42.4000</td>
<td>2</td>
<td>BAW</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.3622</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>2.5149</td>
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</table>

Means with the same letter are not significantly different.
<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>44.83</td>
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<td>BAWSOAK</td>
</tr>
<tr>
<td>A</td>
<td>44.04</td>
<td>2</td>
<td>BW</td>
</tr>
<tr>
<td>A</td>
<td>42.40</td>
<td>2</td>
<td>BAW</td>
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</tbody>
</table>

Anova comparison of glucan composition of 21 kHz for different combination mixtures.

The SAS System

The MEANS Procedure
MixtureID=BAW

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>43.115</td>
<td>0.304</td>
<td>0.7052</td>
<td>0.0924500</td>
</tr>
</tbody>
</table>

MixtureID=BAWSOAK

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>43.265</td>
<td>0.883</td>
<td>2.0429</td>
<td>0.7812500</td>
</tr>
</tbody>
</table>

MixtureID=BW

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>43.720</td>
<td>0.636</td>
<td>1.4556</td>
<td>0.4050000</td>
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</tbody>
</table>
The GLM Procedure

Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID</td>
<td>3</td>
<td>BAW BAWSOAK BW</td>
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</tbody>
</table>

Number of Observations Read 6
Number of Observations Used 6

The GLM Procedure

Dependent Variable: glucan

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.39703333</td>
<td>0.19851667</td>
<td>0.47</td>
<td>0.6666</td>
</tr>
<tr>
<td>Error</td>
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<td>1.27870000</td>
<td>0.42623333</td>
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<td></td>
</tr>
<tr>
<td>Corrected Total</td>
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<td>1.67573333</td>
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<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>R-Square</th>
<th>Coeff Var</th>
<th>Root MSE</th>
<th>glucan Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.236931</td>
<td>1.505455</td>
<td>0.652865</td>
<td>43.36667</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID</td>
<td>2</td>
<td>0.39703333</td>
<td>0.19851667</td>
<td>0.47</td>
<td>0.6666</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
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<td>MixtureID</td>
<td>2</td>
<td>0.39703333</td>
<td>0.19851667</td>
<td>0.47</td>
<td>0.6666</td>
</tr>
</tbody>
</table>
## The GLM Procedure

### Levene's Test for Homogeneity of glucan Variance

ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID</td>
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<td>0</td>
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<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Error</td>
<td>0</td>
<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

![Distribution of glucan](image)

- **F**: 0.47
- **Pr > F**: 0.6666
The GLM Procedure

Duncan's Multiple Range Test for glucan

Alpha 0.05
Error Degrees of Freedom 3
Error Mean Square 0.426233

Number of Means 2 3
Critical Range 2.078 2.085

Means with the same letter are not significantly different.
The GLM Procedure

Tukey's Studentized Range (HSD) Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.426233</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>2.7281</td>
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Tukey Grouping

<table>
<thead>
<tr>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>43.7200</td>
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<td>BW</td>
</tr>
<tr>
<td>43.2650</td>
<td>2</td>
<td>BAWSOAK</td>
</tr>
<tr>
<td>43.1150</td>
<td>2</td>
<td>BAW</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.
Anova comparison of % cellulose digestibility (after 72 hours) of different pretreated combination mixtures for 20 khz frequency.

<table>
<thead>
<tr>
<th>MixtureID</th>
<th>Analysis Variable : cellulosedigestibility</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAW</td>
<td></td>
<td>17.885</td>
<td>0.049497</td>
<td>0.2767541</td>
<td>0.0024500</td>
</tr>
<tr>
<td>BAWSOAK</td>
<td></td>
<td>17.505</td>
<td>0.021213</td>
<td>0.1211837</td>
<td>0.00045000</td>
</tr>
<tr>
<td>BW</td>
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<td>17.685</td>
<td>0.233345</td>
<td>1.3194529</td>
<td>0.0544500</td>
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<td>15.905</td>
<td>0.021213</td>
<td>0.1333744</td>
<td>0.00045000</td>
</tr>
</tbody>
</table>

The GLM Procedure

Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID</td>
<td>4</td>
<td>BAW BAWSOAK BW EC</td>
</tr>
</tbody>
</table>

Number of Observations Read 8
Number of Observations Used 8
The GLM Procedure

Dependent Variable: cellulosedigestibility

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>4.93280000</td>
<td>1.64426667</td>
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</tr>
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<td>Error</td>
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<td>Corrected Total</td>
<td>7</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  cellulosedigestibility Mean
0.988418   0.697061   0.120208   17.24500

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID</td>
<td>3</td>
<td>4.93280000</td>
<td>1.64426667</td>
<td>113.79</td>
<td>0.0003</td>
</tr>
</tbody>
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<table>
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<th>Mean Square</th>
<th>F Value</th>
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<tbody>
<tr>
<td>MixtureID</td>
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<td>1.64426667</td>
<td>113.79</td>
<td>0.0003</td>
</tr>
</tbody>
</table>
### Levene's Test for Homogeneity of Variance

**ANOVA of Squared Deviations from Group Means**

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
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<td>.</td>
</tr>
<tr>
<td>Error</td>
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<td>0</td>
<td>.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### Distribution of Cellulosedigestibility

![Distribution of cellulosedigestibility](image)
The GLM Procedure

Duncan's Multiple Range Test for cellulosedigestibility

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>4</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.01445</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Means</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical Range</td>
<td>.3338</td>
<td>.3411</td>
<td>.3428</td>
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</tbody>
</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
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<td>BAW</td>
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<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>17.6850</td>
<td>2</td>
<td>BW</td>
</tr>
<tr>
<td>B</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>17.5050</td>
<td>2</td>
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</tr>
<tr>
<td>C</td>
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<td>EC</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for cellulosedigestibility

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>4</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.01445</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.75704</td>
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<tr>
<td>Minimum Significant Difference</td>
<td>0.4893</td>
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Means with the same letter are not significantly different.
### Anova comparison of % cellulose digestibility (after 72 hours) of pretreated energy cane bagasse mixture with water and ammonia for various frequencies.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.8850</td>
<td>2</td>
<td>BAW</td>
</tr>
<tr>
<td>A</td>
<td>17.6850</td>
<td>2</td>
<td>BW</td>
</tr>
<tr>
<td>A</td>
<td>17.5050</td>
<td>2</td>
<td>BAWSOAK</td>
</tr>
<tr>
<td>B</td>
<td>15.9050</td>
<td>2</td>
<td>EC</td>
</tr>
</tbody>
</table>

The SAS System

The MEANS Procedure
freq=20

<table>
<thead>
<tr>
<th>Analysis Variable : celldig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>17.8500000</td>
</tr>
</tbody>
</table>

freq=20.5

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>17.4500000</td>
</tr>
</tbody>
</table>

freq=21

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>17.2850000</td>
</tr>
</tbody>
</table>

The GLM Procedure

Class Level Information
### Class Levels Values

| freq | 3   | 20  | 20.5 | 21   |

### Number of Observations Read

- Number of Observations Read: 6

### The GLM Procedure

**Dependent Variable: celldig**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.33763333</td>
<td>0.16881667</td>
<td>20.88</td>
<td>0.0173</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>0.02425000</td>
<td>0.00808333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5</td>
<td>0.36188333</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R-Square</th>
<th>Coeff Var</th>
<th>Root MSE</th>
<th>celldig Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.932989</td>
<td>0.512926</td>
<td>0.089907</td>
<td>17.52833</td>
</tr>
</tbody>
</table>

### Source DF Type I SS Mean Square F Value Pr > F

| freq     | 2  | 0.33763333 | 0.16881667 | 20.88   | 0.0173 |

### Source DF Type III SS Mean Square F Value Pr > F

| freq     | 2  | 0.33763333 | 0.16881667 | 20.88   | 0.0173 |
The GLM Procedure

Levene's Test for Homogeneity of celldig Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>freq</td>
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<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Error</td>
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<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Distribution of celldig

F 20.88
Prob > F 0.0173
The GLM Procedure

Duncan's Multiple Range Test for celldig

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.008083</td>
</tr>
<tr>
<td>Number of Means</td>
<td>2, 3</td>
</tr>
<tr>
<td>Critical Range</td>
<td>0.2861, 0.2871</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.
<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.85000</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>17.45000</td>
<td>2</td>
<td>20.5</td>
</tr>
<tr>
<td>B</td>
<td>17.28500</td>
<td>2</td>
<td>21</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for celldig

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.008083</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>0.3757</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.85000</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>17.45000</td>
<td>2</td>
<td>20.5</td>
</tr>
<tr>
<td>B</td>
<td>17.28500</td>
<td>2</td>
<td>21</td>
</tr>
</tbody>
</table>
Anova comparison of % cellulose digestibility (after 72 hours) of pretreated energy cane bagasse mixture with water (no ammonia) for various frequencies.

The SAS System

The MEANS Procedure
dfreq=20.5k

<table>
<thead>
<tr>
<th>Analysis Variable : celldig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>17.3350000</td>
</tr>
</tbody>
</table>

dfreq=20k

<table>
<thead>
<tr>
<th>Analysis Variable : celldig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>17.9100000</td>
</tr>
</tbody>
</table>

dfreq=21k

<table>
<thead>
<tr>
<th>Analysis Variable : celldig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>17.1700000</td>
</tr>
</tbody>
</table>

The GLM Procedure

<table>
<thead>
<tr>
<th>Class Level Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class Levels Values</td>
</tr>
<tr>
<td>freq</td>
</tr>
</tbody>
</table>

Number of Observations Read  6
Number of Observations Used  6
The GLM Procedure

Dependent Variable: celldig

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.60363333</td>
<td>0.30181667</td>
<td>235.18</td>
<td>0.0005</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>0.00385000</td>
<td>0.00128333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5</td>
<td>0.60748333</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  celldig Mean
0.993662  0.205038  0.035824  17.47167

Source   DF | Type I SS | Mean Square | F Value  | Pr > F |
freq      2  | 0.60363333 | 0.30181667 | 235.18   | 0.0005 |

Source   DF | Type III SS | Mean Square | F Value  | Pr > F |
freq      2  | 0.60363333 | 0.30181667 | 235.18   | 0.0005 |

Distribution of celldig

F  235.10
Pr > F  0.0005
### Levene's Test for Homogeneity of celldig Variance

ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Error</td>
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<td>.</td>
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</tbody>
</table>

![Distribution of celldig](image-url)
The GLM Procedure

Duncan's Multiple Range Test for celldig

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.001283</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Means</th>
<th>2 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical Range</td>
<td>.1140 .1144</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.91000</td>
<td>2</td>
<td>20k</td>
</tr>
<tr>
<td>B</td>
<td>17.33500</td>
<td>2</td>
<td>20.5k</td>
</tr>
<tr>
<td>C</td>
<td>17.17000</td>
<td>2</td>
<td>21k</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for celldig

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.001283</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>0.1497</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.
Anova comparison of % cellulose digestibility (after 72 hours) of pretreated energy cane bagasse mixture with water and ammonia soaked for various frequencies.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.91000</td>
<td>2</td>
<td>20k</td>
</tr>
<tr>
<td>B</td>
<td>17.33500</td>
<td>2</td>
<td>20.5k</td>
</tr>
<tr>
<td>C</td>
<td>17.17000</td>
<td>2</td>
<td>21k</td>
</tr>
</tbody>
</table>
The GLM Procedure

**Class Level Information**

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>freq</td>
<td>3</td>
<td>20.5k 20k 21k</td>
</tr>
</tbody>
</table>

**Number of Observations Read** 6

**Number of Observations Used** 6

The GLM Procedure

Dependent Variable: celldig

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.32110000</td>
<td>0.16055000</td>
<td>60.97</td>
<td>0.0037</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>0.00790000</td>
<td>0.00263333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5</td>
<td>0.32900000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  celldig Mean
0.975988  0.298349  0.051316  17.20000

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>freq</td>
<td>2</td>
<td>0.32110000</td>
<td>0.16055000</td>
<td>60.97</td>
<td>0.0037</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>freq</td>
<td>2</td>
<td>0.32110000</td>
<td>0.16055000</td>
<td>60.97</td>
<td>0.0037</td>
</tr>
</tbody>
</table>
The GLM Procedure

Levene's Test for Homogeneity of celldig Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>freq</td>
<td>0</td>
<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Error</td>
<td>0</td>
<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Distribution of celldig

- F = 60.97
- Prob > F = 0.0037

freq

26.5k 20k 21k

celldig

17.4
17.2
17.0
The GLM Procedure

Duncan's Multiple Range Test for celldig

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.002633</td>
</tr>
<tr>
<td>Number of Means</td>
<td>2</td>
</tr>
<tr>
<td>Critical Range</td>
<td>0.1633 0.1639</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.
The GLM Procedure

Tukey's Studentized Range (HSD) Test for celldig

Alpha 0.05
Error Degrees of Freedom 3
Error Mean Square 0.002633
Critical Value of Studentized Range 5.90958
Minimum Significant Difference 0.2144

Means with the same letter are not significantly different.

Tukey Grouping   Mean   N  freq
A  17.50500  2  20k
B  17.15000  2  20.5k
B  16.94500  2  21k
APPENDIX B
STATISTICAL ANALYSIS FOR CHAPTER 3

Anova comparison of glucan composition of pretreated energy cane bagasse with water and ammonia for different reaction time.

The SAS System

The MEANS Procedure
time=30

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>43.7750000</td>
<td>1.6899852</td>
<td>3.8606173</td>
<td>2.8560500</td>
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</tbody>
</table>

time=45

<table>
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<tr>
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<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45.4400000</td>
<td>1.2727922</td>
<td>2.8010392</td>
<td>1.6200000</td>
</tr>
</tbody>
</table>

time=60

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50.9350000</td>
<td>2.4678027</td>
<td>4.8450038</td>
<td>6.0900500</td>
</tr>
</tbody>
</table>

The GLM Procedure

Class Level Information
Class Levels Values
time 3 30 45 60

Number of Observations Read 6
Number of Observations Used 6
The GLM Procedure

Dependent Variable: glucan

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>56.15523333</td>
<td>28.07761667</td>
<td>7.97</td>
<td>0.0630</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>10.56610000</td>
<td>3.52203333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5</td>
<td>66.72133333</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  glucan Mean
0.841638  4.017213  1.876708  46.71667

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>2</td>
<td>56.15523333</td>
<td>28.07761667</td>
<td>7.97</td>
<td>0.0630</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>2</td>
<td>56.15523333</td>
<td>28.07761667</td>
<td>7.97</td>
<td>0.0630</td>
</tr>
</tbody>
</table>

Distribution of glucan

F 7.97
Prob > F 0.0630
The GLM Procedure

Levene's Test for Homogeneity of glucan Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>0</td>
<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Error</td>
<td>0</td>
<td>0</td>
<td>.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The GLM Procedure

Duncan's Multiple Range Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>3.522033</td>
</tr>
</tbody>
</table>

| Number of Means | 2    | 3    |
| Critical Range  | 5.973 | 5.992 |

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50.935</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>45.440</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>B</td>
<td>43.775</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>3.522033</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>7.8422</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.
<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50.935</td>
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<tr>
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<td>45.440</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>A</td>
<td>43.775</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

Anova comparison of glucan composition of pretreated energy cane bagasse with water (no ammonia) for different reaction time.

The SAS System

The MEANS Procedure

time=30

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32.660</td>
<td>0.8768124</td>
<td>2.6846675</td>
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</table>

time=45

<table>
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<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32.775</td>
<td>0.3323402</td>
<td>1.0140051</td>
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time=60

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<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>34.140</td>
<td>0.8909545</td>
<td>2.6097087</td>
<td>0.7938000</td>
</tr>
</tbody>
</table>
The GLM Procedure

Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
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</thead>
<tbody>
<tr>
<td>time</td>
<td>3</td>
<td>30 45 60</td>
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</tbody>
</table>

Number of Observations Read 6
Number of Observations Used 6

The GLM Procedure

Dependent Variable: glucan

<table>
<thead>
<tr>
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<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>2.71123333</td>
<td>1.35561667</td>
<td>2.43</td>
<td>0.2357</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>1.67305000</td>
<td>0.55768333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5</td>
<td>4.38428333</td>
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<td></td>
</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  glucan Mean
0.618398  2.249908  0.746782  33.19167

<table>
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<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>2</td>
<td>2.71123333</td>
<td>1.35561667</td>
<td>2.43</td>
<td>0.2357</td>
</tr>
</tbody>
</table>

<table>
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<th>F Value</th>
<th>Pr &gt; F</th>
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</thead>
<tbody>
<tr>
<td>time</td>
<td>2</td>
<td>2.71123333</td>
<td>1.35561667</td>
<td>2.43</td>
<td>0.2357</td>
</tr>
</tbody>
</table>
The GLM Procedure

Levene's Test for Homogeneity of glucan Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>Error</td>
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<td>.</td>
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</tbody>
</table>

Distribution of glucan

F 2.43
Pr > F 0.2357
The GLM Procedure

Duncan's Multiple Range Test for glucan

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.557683</td>
<td></td>
</tr>
<tr>
<td>Number of Means</td>
<td>2</td>
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<tr>
<td>Critical Range</td>
<td>2.377</td>
<td>2.384</td>
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</table>
Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>34.1400</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>A</td>
<td>32.7750</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>A</td>
<td>32.6600</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for glucan

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.557683</td>
</tr>
<tr>
<td>Critical Value</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>3.1206</td>
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Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>34.1400</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>A</td>
<td>32.7750</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>A</td>
<td>32.6600</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>
Anova comparison of glucan composition of pretreated energy cane bagasse with water and ammonia soaked for different reaction time.

<table>
<thead>
<tr>
<th>Analysis Variable : glucan</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time=30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>42.6500000</td>
<td>0.7495332</td>
<td>1.7574049</td>
<td>0.5618000</td>
</tr>
<tr>
<td></td>
<td>time=45</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>45.6150000</td>
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<td></td>
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<td>0.7864161</td>
<td>0.1404500</td>
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</tbody>
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The GLM Procedure

<table>
<thead>
<tr>
<th>Class Level Information</th>
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</thead>
<tbody>
<tr>
<td>Class Levels Values</td>
</tr>
<tr>
<td>time</td>
</tr>
<tr>
<td>3 30 45 60</td>
</tr>
</tbody>
</table>

Number of Observations Read 6
Number of Observations Used 6
The GLM Procedure

Dependent Variable: glucan

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>25.33523333</td>
<td>12.66761667</td>
<td>38.05</td>
<td>0.0074</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>0.99870000</td>
<td>0.33290000</td>
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<tr>
<td>Corrected Total</td>
<td>5</td>
<td>26.33393333</td>
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</table>

R-Square  Coeff Var  Root MSE  glucan Mean
0.962076   1.273488   0.576975   45.30667

<table>
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<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>2</td>
<td>25.33523333</td>
<td>12.66761667</td>
<td>38.05</td>
<td>0.0074</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>2</td>
<td>25.33523333</td>
<td>12.66761667</td>
<td>38.05</td>
<td>0.0074</td>
</tr>
</tbody>
</table>
The GLM Procedure

Levene's Test for Homogeneity of glucan Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
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<td>time</td>
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<tr>
<td>Error</td>
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Distribution of glucan

F 38.05
Prob > F 0.0074
The GLM Procedure

Distribution of glucan

<table>
<thead>
<tr>
<th>time</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucan</td>
<td>42</td>
<td>45</td>
<td>48</td>
</tr>
</tbody>
</table>
The GLM Procedure

Duncan's Multiple Range Test for glucan

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha</strong></td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Error Degrees of Freedom</strong></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Error Mean Square</strong></td>
<td>0.3329</td>
<td></td>
</tr>
</tbody>
</table>

| **Number of Means** | 2 | 3 |
| **Critical Range**  | 1.836 | 1.842 |

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>47.6550</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>B</td>
<td>45.6150</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>C</td>
<td>42.6500</td>
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<td>30</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for glucan

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha</strong></td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Error Degrees of Freedom</strong></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Error Mean Square</strong></td>
<td>0.3329</td>
<td></td>
</tr>
<tr>
<td><strong>Critical Value of Studentized Range</strong></td>
<td>5.90958</td>
<td></td>
</tr>
<tr>
<td><strong>Minimum Significant Difference</strong></td>
<td>2.411</td>
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Means with the same letter are not significantly different.
Anova comparison of glucan composition of different non-soaked pretreated sample mixtures for 60 minutes reaction time.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>47.6550</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>A</td>
<td>45.6150</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>A</td>
<td>45.6150</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>B</td>
<td>42.6500</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

The SAS System

The MEANS Procedure
MixtureID=baw

Analysis Variable : glucan

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.9350000</td>
<td>2.4678027</td>
<td>4.8450038</td>
<td>6.0900500</td>
</tr>
</tbody>
</table>

MixtureID=baw,0

Analysis Variable : glucan

<table>
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<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
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<tbody>
<tr>
<td>32.0150000</td>
<td>0.2474874</td>
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MixtureID=bw

Analysis Variable : glucan

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<tr>
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<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.1400000</td>
<td>0.8909545</td>
<td>2.6097087</td>
<td>0.7938000</td>
</tr>
</tbody>
</table>

MixtureID=bw,0

Analysis Variable : glucan

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
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<tbody>
<tr>
<td>25.7400000</td>
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The GLM Procedure

Class Level Information

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<thead>
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<th>Class</th>
<th>Levels</th>
<th>Values</th>
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Number of Observations Read 8
Number of Observations Used 8

The GLM Procedure

Dependent Variable: glucan

<table>
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<tr>
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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>694.6388500</td>
<td>231.5462833</td>
<td>120.45</td>
<td>0.0002</td>
</tr>
<tr>
<td>Error</td>
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<td>1.9223250</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  glucan Mean
0.989052  3.882880  1.386479  35.70750

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
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<tbody>
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ANOVA of Squared Deviations from Group Means

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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<td>.</td>
<td>.</td>
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</table>
The GLM Procedure

Duncan's Multiple Range Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
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<tbody>
<tr>
<td>Error Degrees of Freedom</td>
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<tr>
<td>Error Mean Square</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Means</th>
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</thead>
<tbody>
<tr>
<td>Critical Range</td>
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<td>3.934</td>
<td>3.954</td>
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</tbody>
</table>

Means with the same letter are not significantly different.

Duncan Grouping  | Mean N MixtureID

Means with the same letter are not significantly different.
Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50.935</td>
<td>2</td>
<td>baw</td>
</tr>
<tr>
<td>B</td>
<td>34.140</td>
<td>2</td>
<td>bw</td>
</tr>
<tr>
<td>B</td>
<td>32.015</td>
<td>2</td>
<td>baw,0</td>
</tr>
<tr>
<td>C</td>
<td>25.740</td>
<td>2</td>
<td>bw,0</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>4</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>1.922325</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.75704</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>5.6441</td>
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</tbody>
</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50.935</td>
<td>2</td>
<td>baw</td>
</tr>
<tr>
<td>B</td>
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<td>bw</td>
</tr>
<tr>
<td>B</td>
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<td>2</td>
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</tr>
<tr>
<td>C</td>
<td>25.740</td>
<td>2</td>
<td>bw,0</td>
</tr>
</tbody>
</table>
Anova comparison of glucan composition of different soaked pretreated sample mixtures for 60 minutes reaction time.

<table>
<thead>
<tr>
<th>MixtureID</th>
<th>Analysis Variable: glucan</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>bawsoak</td>
<td></td>
<td>47.655</td>
<td>0.3748</td>
<td>0.7864</td>
<td>0.1405</td>
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<tr>
<td>bwsoak</td>
<td></td>
<td>40.560</td>
<td>0.6223</td>
<td>1.5342</td>
<td>0.3872</td>
</tr>
<tr>
<td>bwsoak,0</td>
<td></td>
<td>38.120</td>
<td>0.2828</td>
<td>0.7419</td>
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<tr>
<td>bwsoak,0</td>
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<td>33.225</td>
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<td>0.8064</td>
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</table>
### Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MixtureID</td>
<td>4</td>
<td>bawsoak, bwsoak, bwsoak,0</td>
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</tbody>
</table>

Number of Observations Read: 8  
Number of Observations Used: 8

### The GLM Procedure

**Dependent Variable: glucan**

<table>
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<tr>
<th>Source</th>
<th>DF</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>216.5985000</td>
<td>72.1995000</td>
<td>204.23</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Error</td>
<td>4</td>
<td>1.4141000</td>
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<td></td>
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<td>Corrected Total</td>
<td>7</td>
<td>218.0126000</td>
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<td></td>
</tr>
</tbody>
</table>

R-Square: 0.993514  
Coeff Var: 1.490548  
Root MSE: 0.594580  
Mean: 39.89000

<table>
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<tr>
<th>Source</th>
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<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
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</thead>
<tbody>
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<td>216.5985000</td>
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</tbody>
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<table>
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<tbody>
<tr>
<td>MixtureID</td>
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<td>216.5985000</td>
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<td>204.23</td>
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</tbody>
</table>
The GLM Procedure

Levene's Test for Homogeneity of glucan Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
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<th>Sum of Squares</th>
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</thead>
<tbody>
<tr>
<td>MixtureID</td>
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<tr>
<td>Error</td>
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<td>0</td>
<td></td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Distribution of glucan

- F = 204.23
- Prob > F < .0001
The GLM Procedure

Duncan's Multiple Range Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>4</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.353525</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Means</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical Range</td>
<td>1.651</td>
<td>1.687</td>
<td>1.696</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.

Duncan Grouping | Mean | N | MixtureID

Means with the same letter are not significantly different.
Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
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</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>47.6550</td>
<td>2</td>
<td>bawsoak</td>
</tr>
<tr>
<td>B</td>
<td>40.5600</td>
<td>2</td>
<td>bawsoak,0</td>
</tr>
<tr>
<td>C</td>
<td>38.1200</td>
<td>2</td>
<td>bwsoak</td>
</tr>
<tr>
<td>D</td>
<td>33.2250</td>
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<td>bwsoak,0</td>
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</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for glucan

<table>
<thead>
<tr>
<th>Alpha</th>
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<tbody>
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<td>4</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.353525</td>
</tr>
<tr>
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<td>5.75704</td>
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<tr>
<td>Minimum Significant Difference</td>
<td>2.4204</td>
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Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
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</thead>
<tbody>
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<td>47.6550</td>
<td>2</td>
<td>bawsoak</td>
</tr>
<tr>
<td>B</td>
<td>40.5600</td>
<td>2</td>
<td>bawsoak,0</td>
</tr>
<tr>
<td>C</td>
<td>38.1200</td>
<td>2</td>
<td>bwsoak</td>
</tr>
<tr>
<td>D</td>
<td>33.2250</td>
<td>2</td>
<td>bwsoak,0</td>
</tr>
</tbody>
</table>
Anova comparison of % cellulose digestibility (after 72 hours) of different non-soaked pretreated sample mixtures along with their untreated controls for 60 minutes reaction time.

The SAS System

The MEANS Procedure
MixtureID=baw

Analysis Variable : cellulosedigestibilty

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>43.6550000</td>
<td>0.6293250</td>
<td>1.4415875</td>
<td>0.3960500</td>
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</tbody>
</table>

MixtureID=baw,0

Analysis Variable : cellulosedigestibilty

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.1800000</td>
<td>0.4666905</td>
<td>1.3265790</td>
<td>0.2178000</td>
</tr>
</tbody>
</table>

MixtureID=bw

Analysis Variable : cellulosedigestibilty

<table>
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<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.6500000</td>
<td>0.6081118</td>
<td>2.8088306</td>
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MixtureID=bw,0

Analysis Variable : cellulosedigestibilty

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<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
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<tbody>
<tr>
<td>21.3850000</td>
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<td>0.0612500</td>
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The GLM Procedure

Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
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<th>Values</th>
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<tbody>
<tr>
<td>MixtureID</td>
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<td>baw baw,0 bw bw,0</td>
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</tbody>
</table>

Number of Observations Read  8
Number of Observations Used   8

The GLM Procedure
Dependent Variable: cellulosedigestibility

<table>
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<tr>
<th>Source</th>
<th>DF</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>712.7158500</td>
<td>237.5719500</td>
<td>909.45</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Error</td>
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<table>
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<th>R-Square</th>
<th>Coeff Var</th>
<th>Root MSE</th>
<th>cellulosedigestibility Mean</th>
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<table>
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</tbody>
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<table>
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Levene's Test for Homogeneity of Variance

ANOVA of Squared Deviations from Group Means

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The GLM Procedure
The GLM Procedure

Duncan's Multiple Range Test for cellulosedigestibility

<table>
<thead>
<tr>
<th></th>
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<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>Alpha</td>
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<td></td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
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<td></td>
</tr>
<tr>
<td>Error Mean Square</td>
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<td></td>
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</tr>
<tr>
<td>Number of Means</td>
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<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Critical Range</td>
<td>1.419</td>
<td>1.450</td>
<td>1.458</td>
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</table>
Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>43.6550</td>
<td>2</td>
<td>baw</td>
</tr>
<tr>
<td>B</td>
<td>35.1800</td>
<td>2</td>
<td>baw,0</td>
</tr>
<tr>
<td>C</td>
<td>21.6500</td>
<td>2</td>
<td>bw</td>
</tr>
<tr>
<td>C</td>
<td>21.3850</td>
<td>2</td>
<td>bw,0</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for cellulosedigestibility

<table>
<thead>
<tr>
<th>Alpha</th>
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<tbody>
<tr>
<td>Error Degrees of Freedom</td>
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<tr>
<td>Error Mean Square</td>
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</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.75704</td>
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<tr>
<td>Minimum Significant Difference</td>
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<td>baw,0</td>
</tr>
<tr>
<td>C</td>
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<td>bw</td>
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<tr>
<td>C</td>
<td>21.3850</td>
<td>2</td>
<td>bw,0</td>
</tr>
</tbody>
</table>
Anova comparison of % cellulose digestibility (after 72 hours) of different soaked pretreated sample mixtures along with their untreated controls for 60 minutes reaction time.

<table>
<thead>
<tr>
<th>MixtureID</th>
<th>Analysis Variable : cellulosedigestibility</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
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The GLM Procedure

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>MixtureID</td>
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<td>bawsoak bawsoak, bwsoak bwsoak,0</td>
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</table>

Number of Observations Read  8
The GLM Procedure

Dependent Variable: cellulosedigestibility

<table>
<thead>
<tr>
<th>Source</th>
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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
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</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>490.7234000</td>
<td>163.5744667</td>
<td>440.96</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Error</td>
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<table>
<thead>
<tr>
<th>R-Square</th>
<th>Coeff Var</th>
<th>Root MSE</th>
<th>cellulosedigestibility Mean</th>
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</thead>
<tbody>
<tr>
<td>0.996985</td>
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<table>
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</table>
The GLM Procedure

Levene's Test for Homogeneity of cellulosedigestibility Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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</thead>
<tbody>
<tr>
<td>MixtureID</td>
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</tr>
<tr>
<td>Error</td>
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<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Distribution of cellulosedigestibility

F  440.96
Prob > F  <.0001
The GLM Procedure

Duncan's Multiple Range Test for cellulosedigestibility

<table>
<thead>
<tr>
<th>Alpha</th>
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</tr>
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<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>4</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.37095</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Means</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical Range</td>
<td>1.691</td>
<td>1.728</td>
<td>1.737</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.

Duncan Grouping | Mean | N | MixtureID
Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>43.3200</td>
<td>2</td>
<td>bawsoak</td>
</tr>
<tr>
<td>B</td>
<td>37.9700</td>
<td>2</td>
<td>bawsoak,0</td>
</tr>
<tr>
<td>C</td>
<td>28.6800</td>
<td>2</td>
<td>bwsoak,0</td>
</tr>
<tr>
<td>D</td>
<td>23.2100</td>
<td>2</td>
<td>bwsoak</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for cellulosedigestibility

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
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</tr>
<tr>
<td>Error Mean Square</td>
<td>0.37095</td>
</tr>
<tr>
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<td>Minimum Significant Difference</td>
<td>2.4794</td>
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</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>MixtureID</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>43.3200</td>
<td>2</td>
<td>bawsoak</td>
</tr>
<tr>
<td>B</td>
<td>37.9700</td>
<td>2</td>
<td>bawsoak,0</td>
</tr>
<tr>
<td>C</td>
<td>28.6800</td>
<td>2</td>
<td>bwsoak,0</td>
</tr>
<tr>
<td>D</td>
<td>23.2100</td>
<td>2</td>
<td>bwsoak</td>
</tr>
</tbody>
</table>
Anova comparison of % cellulose digestibility (after 72 hours) of pretreated energy cane bagasse with water and ammonia for different reaction time.

<table>
<thead>
<tr>
<th>Time</th>
<th>Analysis Variable: celldig</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td></td>
<td>0.350</td>
<td>0.014</td>
<td>4.0406102</td>
<td>0.000200000</td>
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<tr>
<td>45</td>
<td></td>
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<td>0.014</td>
<td>3.288687</td>
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The GLM Procedure

Class Level Information

<table>
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<tr>
<th>Class Levels</th>
<th>Values</th>
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<tbody>
<tr>
<td>time</td>
<td>30 45 60</td>
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</tbody>
</table>

Number of Observations Read 6
Number of Observations Used 6
The GLM Procedure

Dependent Variable: celldig

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.00973333</td>
<td>0.00486667</td>
<td>36.50</td>
<td>0.0078</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>0.00040000</td>
<td>0.00013333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5</td>
<td>0.01013333</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  celldig Mean
0.960526   2.839428   0.011547   0.406667

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>2</td>
<td>0.00973333</td>
<td>0.00486667</td>
<td>36.50</td>
<td>0.0078</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
<tr>
<td>time</td>
<td>2</td>
<td>0.00973333</td>
<td>0.00486667</td>
<td>36.50</td>
<td>0.0078</td>
</tr>
</tbody>
</table>

Distribution of celldig
The GLM Procedure

Levene's Test for Homogeneity of celldig Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
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<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Error</td>
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<td>0</td>
<td>.</td>
<td>.</td>
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</tbody>
</table>

The GLM Procedure

Distribution of celldig

![Distribution of celldig](image)
The GLM Procedure

Duncan's Multiple Range Test for celldig

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.000133</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Means</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical Range</td>
<td>0.03675</td>
<td>0.03687</td>
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</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
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</thead>
<tbody>
<tr>
<td>A</td>
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<td>60</td>
</tr>
<tr>
<td>A</td>
<td>0.43000</td>
<td>2</td>
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</tr>
<tr>
<td>A</td>
<td>0.43000</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>B</td>
<td>0.35000</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for celldig

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.000133</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
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</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.44000</td>
<td>2</td>
<td>60</td>
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</table>
Means with the same letter are not significantly different.

Tukey Grouping

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Mean</th>
<th>N</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>0.43000</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>0.35000</td>
<td>2</td>
</tr>
</tbody>
</table>

Anova comparison of % cellulose digestibility (after 72 hours) of pretreated energy cane bagasse with water (no ammonia) for different reaction time.

The SAS System

The MEANS Procedure
time=30

Analysis Variable : celldig
Mean Std Dev Coeff of Variation Corrected SS
0.1700000 0 0 0

time=45

Analysis Variable : celldig
Mean Std Dev Coeff of Variation Corrected SS
0.2050000 0.0070711 3.4493014 0.000050000

time=60

Analysis Variable : celldig
Mean Std Dev Coeff of Variation Corrected SS
0.2250000 0.0070711 3.1426968 0.000050000
The GLM Procedure

Class Level Information

Class Levels Values
time 3 30 45 60

Number of Observations Read 6
Number of Observations Used 6

The GLM Procedure

Dependent Variable: celldig

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.00310000</td>
<td>0.00155000</td>
<td>46.50</td>
<td>0.0055</td>
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<td>0.00003333</td>
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<tr>
<td>Corrected Total</td>
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<td>0.00320000</td>
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</table>

R-Square Coeff Var Root MSE celldig Mean
0.968750 2.886751 0.005774 0.200000

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
<tr>
<td>time</td>
<td>2</td>
<td>0.00310000</td>
<td>0.00155000</td>
<td>46.50</td>
<td>0.0055</td>
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<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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</thead>
<tbody>
<tr>
<td>time</td>
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<td>0.00310000</td>
<td>0.00155000</td>
<td>46.50</td>
<td>0.0055</td>
</tr>
</tbody>
</table>
The GLM Procedure

Levene's Test for Homogeneity of Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
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<tbody>
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<tr>
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</table>
The GLM Procedure

Duncan's Multiple Range Test for celldig

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
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<tr>
<td>Error Mean Square</td>
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<table>
<thead>
<tr>
<th>Number of Means</th>
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<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical Range</td>
<td>.01837</td>
<td>.01843</td>
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</table>

Means with the same letter are not significantly different.

Duncan Grouping | Mean | N | time

---

The GLM Procedure

Distribution of celldig
<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.225000</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>B</td>
<td>0.205000</td>
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</tr>
<tr>
<td>C</td>
<td>0.170000</td>
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</table>

Means with the same letter are not significantly different.

The GLM Procedure

Tukey's Studentized Range (HSD) Test for celldig

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
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<tr>
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<tr>
<td>Error Mean Square</td>
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<tr>
<td>Minimum Significant Difference</td>
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<table>
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<tr>
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<th>time</th>
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</thead>
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<tr>
<td>A</td>
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<td>2</td>
<td>30</td>
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</table>

Means with the same letter are not significantly different.
Anova comparison of % cellulose digestibility (after 72 hours) of pretreated energy cane bagasse with water and ammonia soaked for different reaction time.

The SAS System

The MEANS Procedure

time=30

Analysis Variable : celldig

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2150000</td>
<td>0.0070711</td>
<td>3.288687</td>
<td>0.000050000</td>
</tr>
</tbody>
</table>

time=45

Analysis Variable : celldig

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3850000</td>
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<td>1.836410</td>
<td>0.000050000</td>
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</tbody>
</table>

time=60

Analysis Variable : celldig

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<th>Std Dev</th>
<th>Coeff of Variation</th>
<th>Corrected SS</th>
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The GLM Procedure

Class Level Information

<table>
<thead>
<tr>
<th>Class Level Information</th>
<th>Class Levels</th>
<th>Values</th>
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</thead>
<tbody>
<tr>
<td>time</td>
<td>3 30 45 60</td>
<td></td>
</tr>
</tbody>
</table>

Number of Observations Read 6
Number of Observations Used 6
The GLM Procedure

Dependent Variable: celldig

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
<tr>
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<td>0.02846667</td>
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<td>0.0001</td>
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<td>0.00005000</td>
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</tr>
<tr>
<td>Corrected Total</td>
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<td>0.05708333</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square  Coeff Var  Root MSE  celldig Mean
0.997372   2.029972   0.007071   0.348333

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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</thead>
<tbody>
<tr>
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<td>0.02846667</td>
<td>569.33</td>
<td>0.0001</td>
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</tbody>
</table>

<table>
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<td>0.05693333</td>
<td>0.02846667</td>
<td>569.33</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
The GLM Procedure

Levene's Test for Homogeneity of celldig Variance
ANOVA of Squared Deviations from Group Means

<table>
<thead>
<tr>
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<th>Pr &gt; F</th>
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</thead>
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<td>0</td>
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<td>.</td>
</tr>
<tr>
<td>Error</td>
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<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
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</tbody>
</table>

Distribution of celldig

<table>
<thead>
<tr>
<th>V</th>
<th>0.45</th>
<th>0.40</th>
<th>0.35</th>
<th>0.30</th>
<th>0.25</th>
<th>0.20</th>
</tr>
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</table>
| F | 569.33 | Prob > F | 0.0001 |}

145
The GLM Procedure

Duncan's Multiple Range Test for celldig

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
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<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
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<td>0.00005</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Means</td>
<td>2 3</td>
</tr>
<tr>
<td>Critical Range</td>
<td>.02250 .02258</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.445000</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>B</td>
<td>0.385000</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>C</td>
<td>0.215000</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

The GLM Procedure

Tukey's Studentized Range (HSD) Test for celldig

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of Freedom</td>
<td>3</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>0.00005</td>
</tr>
<tr>
<td>Critical Value of Studentized Range</td>
<td>5.90958</td>
</tr>
<tr>
<td>Minimum Significant Difference</td>
<td>0.0295</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.
<table>
<thead>
<tr>
<th>Tukey Grouping</th>
<th>Mean</th>
<th>N</th>
<th>time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.445000</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>B</td>
<td>0.385000</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>C</td>
<td>0.215000</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

**SAS coding for glucan comparison**

```sas
dm'log;clear;output;close';
data E;
input MixtureID$ FREQ$ specimen$ GLUCAN;
cards;
  BAW 21 1 43.33
  BAW 21 2 42.9
  BAW 20.5 1 42.26
  BAW 20.5 2 42.54
  BAW 20 1 43.25
  BAW 20 2 41.54;
ods rtf file='C:\Users\hchall1\Desktop\sas_BAW GLUCAN FREQ comparison.rtf';
proc sort data=E;
  by FREQ;
run;
proc means data=E mean std cv css;
  var GLUCAN;
  by FREQ;
```
run;
proc glm data=E ;
class FREQ;
model GLUCAN=FREQ;
means FREQ/hovtest=levene duncan tukey;
run;
ods rtf close;
quit;

SAS coding for cellulose digestibility comparison

dm'log;clear;output;clear';
data E;
input MixtureID$ time$ specimen$ cellulosedigestibilty;
cards;
baw  60 1  44.1
baw  60 2  43.21
baw,0 60 1  35.51
baw,0 60 2  34.85
bw  60 1  22.08
bw  60 2  21.22
bw,0 60 1  21.21
bw,0 60 2  21.56;
ods rtf file='C:\Users\hchall1\Desktop\sas_time cellulosedigestibilty comparison.rtf';
proc sort data=E;
by MixtureID;
run;
proc means data=E mean std cv css;
var cellulosedigestibility;
by MixtureID;
run;
proc glm data=E;
class MixtureID;
model cellulosedigestibility=MixtureID;
means MixtureID/hovtest=levene duncan tukey;
run;
ods rtf close;
quit;
ods html close; /* close previous */
ods html; /* open new */
APPENDIX C
PRETREATMENT PROCESSING TEMPERATURE EVOLUTION FOR VARIOUS COMBINATION MIXTURE SAMPLES AT DIFFERENT REACTION

B:W, 60 Min, 200 W

B:W – Biomass:Water

(i)

B:W, 30 Min, 200 W

B:W – Biomass:Water

(ii)
B:A:W – Biomass:Ammonia:Water

(iii)

B:A:W – Biomass:Ammonia:Water

(iv)
B:W SOAK, 30 Min, 200 W

B:W – Biomass: Water

(v)

B:W SOAK, 45 Min, 200 W

B:W – Biomass: Water

(vi)
APPENDIX D
ULTRASONIC PRETREATMENT PICTURES

Overall picture of ultrasonic set up

Overall picture of ultrasonic set up in mineral oil
VITA

Niyaz Ahamed Methrath Liyakathali was born in India. He received his Bachelor of Engineering degree in Electronics and Instrumentation Engineering in June 2008 from Sri Ramakrishna Engineering College (Anna University), India. Niyaz Ahamed started his Dual Masters of Science program in Electrical Engineering and Biological & Agricultural Engineering at Louisiana State University in Fall 2011. In December 2013, he received his first master degree in Electrical Engineering and in May 2014, he expects to receive his second master degree in Biological & Agricultural Engineering with a concentration in bioprocessing & bioenergy.