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An Ionic Liquid Pretreatment System of *Digitaria sanguinalis*

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Abstract

The snowballing demands for cleaner fuel from the transport and industrial sectors has made many believe that biofuel production is the panacea. Is E85 (a laboratory made ethanol based fuel) the cure for the world's dependence on fossil fuels? A keen look exposes a more critical conundrum; the production of E85 destroys human food. With 1 in 8 people suffering from chronic undernourishment in 2010-2012,[1] this production is not justifiable. Biofuels are produced through the break down of plants' cellulosic components (lignocellulose LC) into glucose and then into ethanol. However, two main challenges remain: 1) finding a cheap, reliable and non-food source of LC; i.e. having high cellulose content, and 2; developing a cheap, clean, and reliable conversion/pre-treatment system. Dissolution of cellulosic material in ionic liquids, or ILs (a unique class of solvents) has been reported to make the material susceptible to chemical attack by various reagents/catalysts/acids. Digitaria Sanguinalis (hairy crabgrass, a weed) is reported to have high cellulose content.[2] In this research, crabgrass was pre-treated with a series of imidazolium ionic liquids, for 3, 6, and 9 hours followed by acid hydrolysis. The results attained so far, including those of glucose and total reducing sugar quantification work are presented and discussed.

1 "World hunger poverty facts and statistics." *Worldhunger.org*. 27 July 2013. 21 Mar. 2014. Web.

2 Ogden, R. (2003) "Nutritive value of crabgrass harvested on seven dates in northern Arkansas." *Arkansas Animal Science Department Report* AAES Research series 509: 119.

Introduction

The production of a non food-stock source of biofuels is a multifaceted project. The goal of this project is attaining a method that efficiently uses Ionic Liquids to increase the yield of glucose and eventually ethanol. Ionic liquids have the capability to dissociate celluloses and hemicelluloses from lignin as well as assist in the degradation of the crystalline structure of the cellulose. This is achieved by altering the structure of the biomass, which allows for a higher degree of permeability to acid, ergo assisting the acid hydrolysis process.[3]

Cellulose lignin and hemicellulose are normally associated with one another in a mesh-like fashion. When treated with an acid reagent, this mesh conformation impedes the biomass from undergoing acid hydrolysis. The ILs are used to break down this mesh, providing a favorable yield when treated with an acid reagent.[4] Properties of these Ionic liquids include high hydrogen bonding basicity (which allows the IL to start to dissolve cellulose), as well as low vapor pressure and high affinity for water. Treatment with these ILs will break down the plant material into its three main components: Lignin, Cellulose and Hemicellulose. Figure 1 displays the putative effects of the ionic liquids on the plant mass.[5]

3 Chiaramonti, D. (2012) "Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method." *Biomass and Bioenerg* 46: 25-35.

4 Hsu, T. (1980) "Alcohol from Cellulose." *Chem Technol* 10(5): 315–19.

5 Wang, H. (2012)"Ionic Liquid Processing of cellulose" *Chem Soc* 41: 1519-1537.

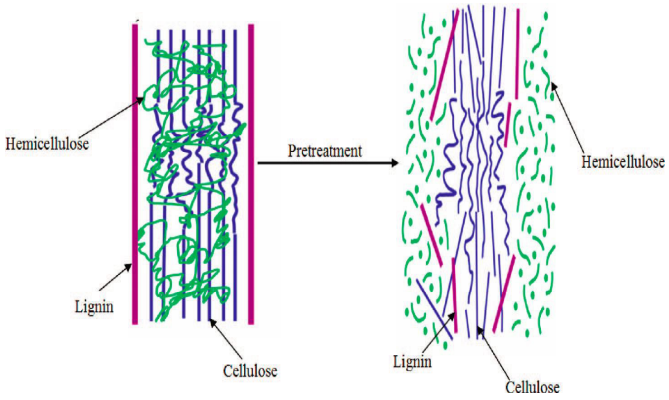

Figure 1: Putitive effects of the pretreatment method

There are myriad options available when it comes to pre-treatment agents. If one decides to delve into the world of ionic liquids, there are still a wide variety of products available. This is why pursuit of an ideal pre-treatment system is necessary. The system that was chosen was Ionic liquid pre-treatment, the reagents purchased from Sigma Aldrich.

The three ILs that were chosen consisted of different carbon side chain lengths, which have not shown a significant difference in the pretreatment effect.

1-Ethyl-3-methylimidazolium chloride, which will be referred to as [E(mim)] product number 30764. 1-hexyll-3-methylimidazolium chloride, which will be referred to as [H(mim)], product number 727954.1-Butyl-3-methylimidazolium chloride, product number 38899 which will now be referred to as [B(mim)] are the pretreatment ILs that where chosen. Figures 2, 3, and 4 show these respectively. Images via the Sigma website sales page.

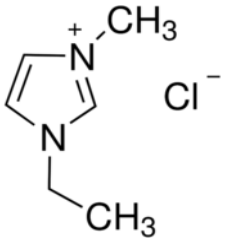

Figure 2: 1-ethyl-3-methylimidazolium chloride [E(mim)]

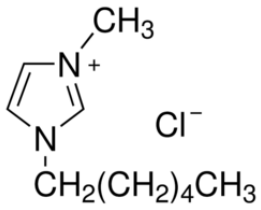

Figure3: 1-hexyll-3-methylimidazolium chloride [H(mim)]

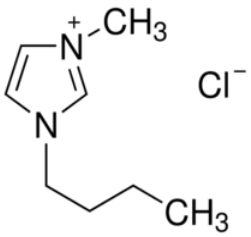

Figure 4: 1-Butyl-3-methylimidazolium chloride [B(mim)]

Another facet of this project was finding a non-food source of biofuel. The answer to this came from a household nuisance, the common crabgrass/ or hairy crabgrass Digitaria Sanguinalis. This plant was harvested in order to provide the source of celluloses and hemicelluloses. This species is very common in residential areas, and seems to pester many residents' lawns, so the harvest of many of these grasses was much appreciated. This grass was chosen based on its cellulose content as well as its lack of utilization as a food source in the northeastern part of America.[6]

The last part of this operation was finding a suitable test for reducing sugar levels. This came from many angles. The spectral perspective, a DNS[7] reagent was reacted with the sample, and read through a spectrophotometer, correlated back to a standard curve giving reduced sugar levels. A glucose refractometer was also used to give reducing sugar concentrations after being blanked with the supernatant. Benedict's Solution was used qualitatively and gave a positive test for reducing sugars. SEM images were also taken to view structural differences responding to pretreatment.

6 Vancov, T. (2012) "Use of ionic liquids in converting lignocellulosic material to biofuels" *Renew Energ*. 45: 1-6.

Procedure

One kilogram of the Digitaria sample was collected from a residential plot and prepped to undergo pre-treatment.

In 30-gram portions the sample (as a whole) was washed with deionized water in a 1L beaker; the water was then removed. This process was repeated until the soil surrounding the Digitaria was removed completely. A standard kitchen blender was then used on a high function level for about one minute to break up the Digitaria, in order to increase the surface area. The samples were then strained and washed in a fishnet to remove any impurities. This sample was then dried in an oven at about 70 degrees Celsius for at least three days (Figure 5). The sample lost most of its mass, but the dry sample gave a more consistent weight to volume ratio. 0.25 grams of these samples were then weighed out and placed in, alone of thirty, 50 mL Erlenmeyer flasks. Next, 2.5 grams of [E(mim)] was placed in nine of the flasks. 2.5 grams of [H(mim)] were placed in another nine. Lastly, 2.5 grams of [B(mim)] were placed in another nine flasks. Three of the last six flasks had only the crabgrass component but did not receive any pre-treatment agent. Three more flasks were prepared containing pure cellulose instead of crabgrass, which would also not receive pre-treatment. (Figure 6)

Figure 5: The dried sample

Figure 6: The samples prepared with Ionic Liquids before heating

The samples were named prior to the addition of the specific IL. Each sample was named after its ionic liquid component and the time that the sample would undergo pre-treatment in hours, followed by the time the sample would undergo acid hydrolysis in 3-hour intervals, such as hexyl3-6 referring to hexyl as the ionic liquid, 3 as the length of pretreatment, and 6 as the length of hydrolyses. This provided 27 samples with varying times of IL pre-treatment as well as acid time in the acid hydrolysis stage per IL. Mineral oil was then heated in crystallizing dishes to a temperature of around 70-80 degrees Celsius for about a half hour and then all of the samples were added. In three-hour intervals, three samples from each of the ionic liquids were removed from heat. After nine hours all of the samples were removed from

heat. These samples where now placed through four rounds of centrifugation where 10mL of water was added, agitated, and then centrifuged out removing the Ionic liquid content. The supernatant from the last round of centrifugation was saved and used to blank for DNS and Refractometry tests, so that the possible residual Ionic liquid would not skew the data. The next day 3 mL of 5% HBr was added to all 33 samples and they were placed in the mineral oil at 70-80 degrees and removed one sample from each of the groups every three hours. As soon as these samples were removed, they were neutralized with eqimolar amount of NaOH and left to cool for another day.

Thirty-three filtering apparatuses were prepared in Pasteur pipettes with the tips removed. The pipettes were filled wool, activated charcoal, then a thin layer of sand. The samples were then added into the filter apparatus at approximately 1 mL intervals until there was no liquid portion left of the sample. The solid portion of the sample was then saved for possible future experimentation—to try and remove the IL content. At the end of this process, 33 samples of approximately 4 mL each were collected for data analysis.

A DNS solution was prepared using cited methods.[7] This solution is sensitive to reducing sugars. A standard curve was prepared with known reducing sugar concentrations and ionic liquid concentrations. This curve was used to help assay the concentrations of the samples themselves. 0.250 mL of the sample as well as 0.075 mL DNS and 2.175 mL deionized water was added to a centrifuge tube and then heated for 15 min at 90 degrees Celsius, then placed directly in a ice bath until reaching room temperature. The sample was then removed and added to a cuvette, run though a spectrophotometer reading at the 540 nm—the specific wavelength where DNS absorption is the highest. This was then correlated back to the standard curve. Both the dilution factor and the total sample size itself were factored in, allowing for the reducing sugar content of the whole sample to be assessed.

Also, a qualitative test was run using a prepared Benedict's Solution; this is a test for the presence of reducing sugars. This was preformed using 0.5 mL of the samples as well as 0.5 mL Benedicts Solution that were reacted in 2 mL of DI water.

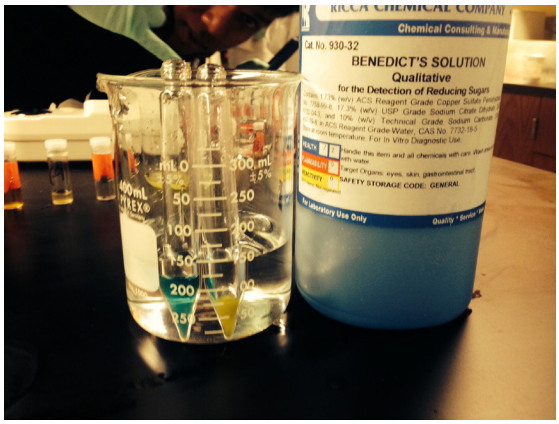

Figure 7: Results of the Benedicts solution

SEM images of the samples prior to treatment, as well as [after] nine hours of treatment were preformed. In order to prepare the samples, the samples were washed in DI water and centrifuged five times and then dried in an oven for one week at 80 degrees centigrade. The dry samples were then gold-coated, placed on stubs, and used for the imaging.

A glucose refractometer was used to help assay the reducing sugar content of the sample. There was a problem with these readings, due to a major bias by ionic liquid to the refract meter. Therefore, the samples were blanked with the final supernatant of the centrifugation prior to reading. The data was not correlated back to the concentration because it is glucose, not reducing sugar specific. Therefore, the readings were used as data themselves to analyze differences in glucose levels.

7 Shengdong, Z. (2006) "Dissolution of cellulose with ionic liquids and its application: a mini review" *Green Chem*, 8: 325-32.

Results

The reducing sugar levels were calculated by the standard curve of the DNS reagent. This allowed for nine samples for each time period, as each sample was assayed by DNS. Cellulose itself was run as a blank and showed no considerable activity when reacted with the DNS reagent. The reducing sugar content for the three times (3 hours, 6 hours, and 9 hours) are shown below in figures 8, 9, and 10. The samples showed medians of 19.634 mg, 21.676 mg and 28.182 mg respectively. However, when significance tests were run, such as two sample t-tests and an ANOVA, the pre-treatment method had produced a significant change between the 3 and 9-hour samples, with a p value of .012 between the 3-hour and the 9-hour samples.

The differences between the Ionic liquids themselves proved to be non-significant, all yielding an average of around 22 mg.

The results of the Benedicts solution showed all of the samples changing from blue to different shades of green. This is a positive on a qualitative level for reducing sugars.

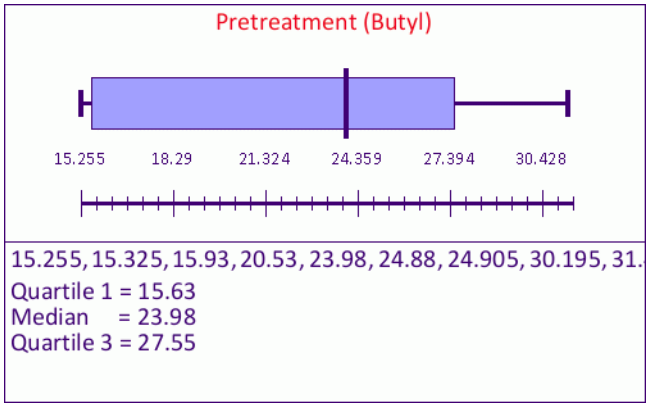

Figure 8: Reducing sugar content in 3-hour samples by DNS test.

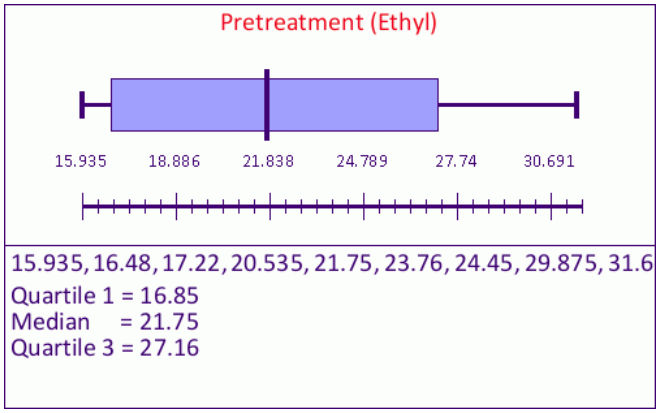

Figure 9: Reducing sugar content in 6-hour samples by DNS test

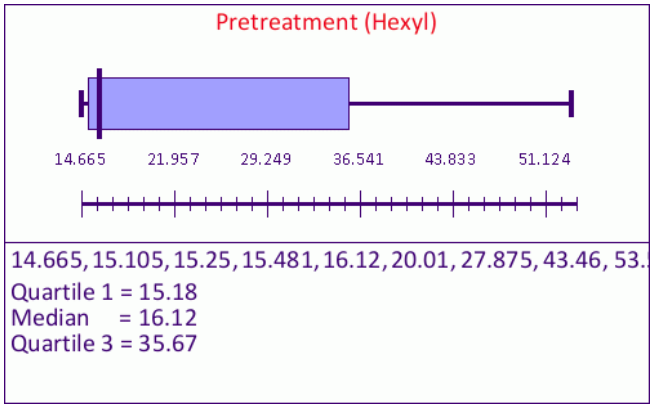

Figure 10: Reducing sugar content in the 9-hour samples by DNS test

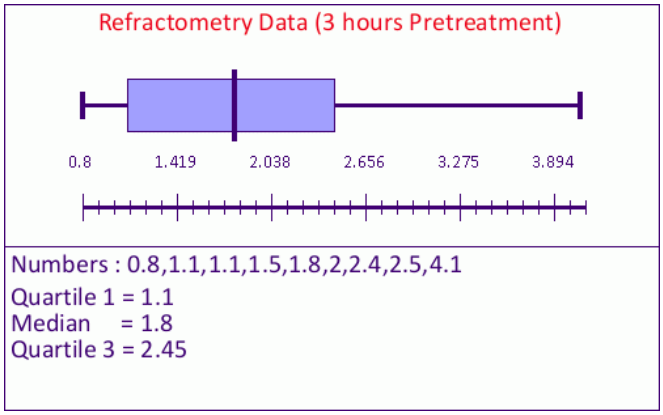

Figure 11: Refractometry Readings

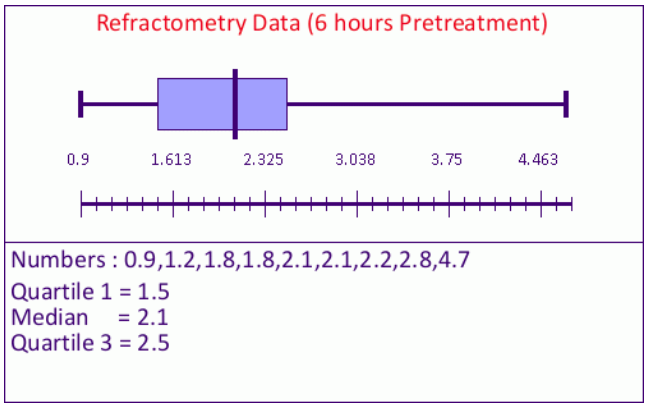

Figure 12: Refractometry Readings

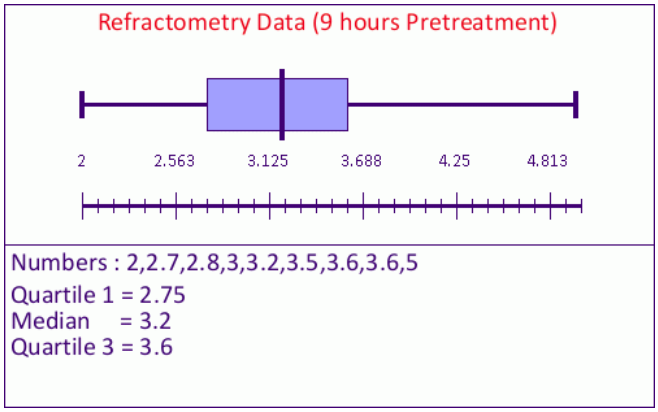

Figure 13: Refractometry Readings

When the samples were assayed for differences in reducing sugar levels by refractometry, the putative effects of the ILs were confirmed. The samples for 3, 6,and 9 hours of pretreatment had readings of, 1.92, 2.17, and 3.27 respectively. The data proved to be significant between 3 and 6 hour and 3 and 9 hours with p values of .031, and .009 respectively. Therefore, although the data is not representative of all of the reducing sugars in the solution, if the ratios of non-reducing sugars to glucose are consistent between the samples, then it is safe to assume that the longer length of pretreatment lead to a higher yield of glucose.

SEM images were captured and searched for morphological differences. Figures 14, 15, and 16 below show the SEM. Figure 14 shows sample prior to pretreatment. Figures 15 and 16 show samples after nine hours of pretreatment.

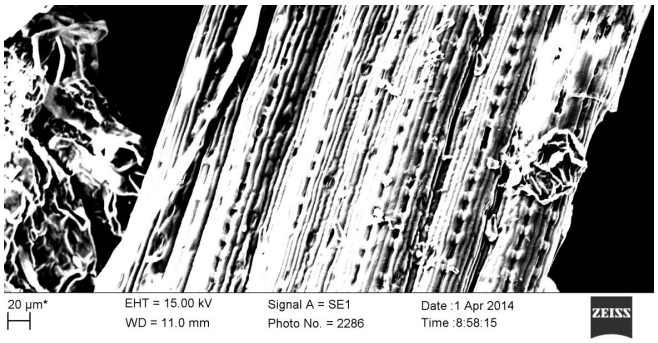

Figure 14: Sample after 9 hours of heating.

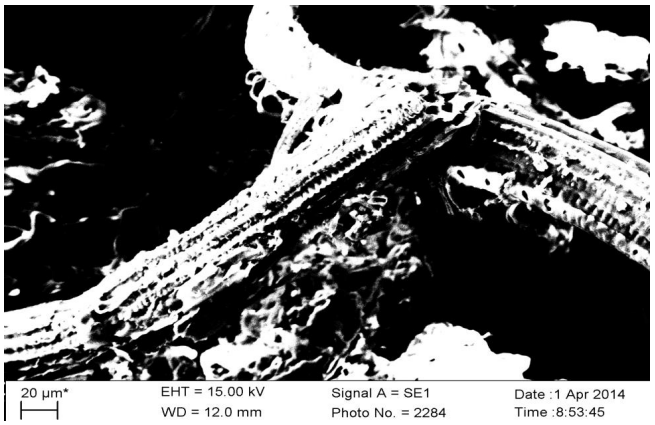

Figure 15: Sample after 9 hours of pretreatment with [H(mim)]

Figure 16: Sample after 9 hours pretreatment with [E(mim)]

Discussion

Pre-treatment with ILs was not only shown to increase the yield of reducing sugars that can be isolated, but also morphologically alter the sample themselves. At this time, ILs are quite expensive and their yield of reducing sugars is far from enough to justify their use for everyday production. This may seem like a problem, but the fact that there are other options, even though not yet completely available, helps to refute the notion that corn and other food-based sources of fuel are the only option. Although crabgrass is still used in many parts of the world as a food-source, in America it is looked at with general distaste. With a source of reducing sugars, the process of enzymatic fermentation to ethanol is relatively simple, cheap, and efficient. In a more environmentally-conscious perspective, the fuel burned becomes CO2, which gets incorporated into the sugars that make more fuel, alleviating the concerns of those who are concerned with the climate changes. Therefore, bio-fuel is much better than having to use sources of energy such as coal and oil. However, this pre-treatment system is far from being economically fit for a large-scale production of ethanol. Acquiring more efficient ways to reduce the amount of ionic liquids used, as well as designing better methods of recycling the compounds for future use, would be necessary for the use of this model on a larger scale. With that said, the pretreatment system did have the effects that were expected. By increasing the yield of reducing sugars, the yield of ethanol will subsequently increase when the sugars are converted to ethanol by fermentation down the line.

Future Work

The enzymatic production of ethanol is the most obvious next step in this procedure. From the data obtained, the production of ethanol from the samples should be a simple and cost-efficient process. Looking into various strains of yeast to find the highest yielding organism is one idea for future work. In another similar field, the production of ethanol directly from crops themselves through direct association of the yeast to the photosynthesizing plant themselves, is also an area of curiosity.

Lastly, algal research is an area of bio-fuel production that is blooming. The prospect of using the algae as the source of cellulose and lignocellulose would have a variety a benefits compared to the plants that are used for biofuel production today. The production of biofuel from non-food stock sources is a field of study that is rapidly changing. There are many possible routes one could go with the resources that we have today.