

# Alfalfa hay as non-human feedstock for second generation biofuels: Hope or hoax? Part II

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## Abstract:

The excessive use of fossil fuels has negatively impacted the environment, because of greenhouse gas emissions. Researchers are now looking to counterbalance the damage which has been done to the Earth after centuries of greenhouse gas emissions. Potential alternatives to fossil fuels include biofuels, an overlooked and cost-effective source of energy. Efforts have been focused on the production of first-generation biofuels, fuels attained from human food sources, such as corn. First-generation biofuels have been linked to the increase in food prices, which negatively impacts many developing countries. Our research is geared towards the production of second generation biofuels, which rely on non-human food sources, specifically alfalfa hay. Second generation biomasses, such as alfalfa hay, are ideal as biofuel feedstock, because they are cheap, and have the power to constrain greenhouse gas emissions, while not taking away major human food sources or causing competition for new land. For this research project, the ionic liquid 1-butyl-3-methylimidazolium chloride was used in the pretreatment of the alfalfa hay, in order to produce glucose.

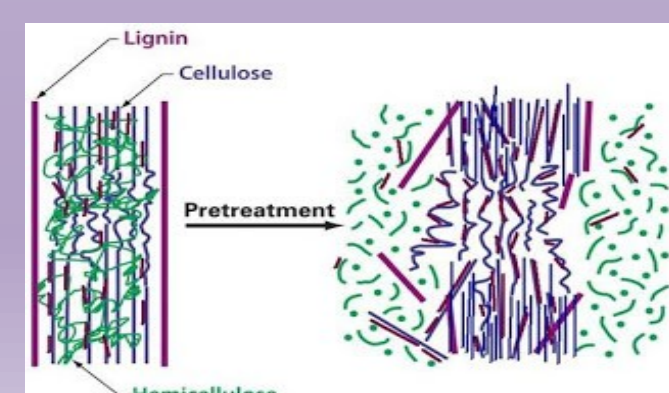
## Introduction :

Biomass is renewable organic matter which can be used as a source of fuel. However, the production of biofuels has mainly been derived from crops such as corn and sugarcane. Biofuels derived from food sources, such as rice or bread, are high in carbohydrates; these are known as first-generation biofuels. Concern has risen regarding the use of food sources for fuel, because it threatens food supplies while increasing the price of food products. Limitations to the use of first generation biofuels also include the competition for land, as well as high costs which are necessary to compete with the production and processing of petroleum products. As an alternative, research has turned to second-generation biofuels, which acquire ethanol from biomass, such as alfalfa hay. Utilizing alfalfa hay is advantageous, because it is a leftover product that is often wasted. Hay has the power to curb greenhouse gas emissions, while not taking away a major food source. Since the growth of hay would already be on pre-existing land, there would not be any competition for new land. Cellulose in these second generation crops offers benefits, such as abundance, diversity and low cost.

Second generation crops involve bioethanol production. These crops are made from lignocellulose, the inedible part of the plant's cell wall, which is made of lignin and cellulose. Cellulose and hemicellulose can be converted into sugars such as glucose and xylose, which can then be fermented into ethanol. Despite the potential benefits, research into the conversion of biomass to liquid fuel has shown to be problematic. Ionic liquids have been used in the process as a form of pretreatment, in order to initiate the breakdown of biomass to cellulose, lignin, and hemicellulose. The goal of breaking down the biomass is to obtain the cellulose and hemicellulose, which can then be converted into glucose, and to eliminate lignin. Hemicellulose is a polysaccharide found in plant cells; it is known to toughen cell walls when interacting with cellulose and, at times, lignin. Cellulose is an insoluble substance, containing many chains of glucose monomers; it is found in the cell walls of plants and vegetable fibers. Cellulosic biomass is an abundant biological energy source. Lignin refers to an aromatic polymer made up of a heterogeneous mix of polymers which plays an important role in the formation of cell walls.

Ionic liquids are salts in which the ions are not coordinated very well, resulting in them being in the liquid state below certain temperatures. They can be useful in synthesis and catalysis. The benefits of using ionic liquids includes the prevention of the emission of VOCs, a major source of environmental pollution. Ionic liquids are also useful, because they can help to maximize yield.

Figure 1. Impact of pretreatment on biomass



## Procedure

### Biomass Preparation

The alfalfa hay was purchased from Rebel Brewer™, TN. The hay was then washed, and placed in the oven to dry. Using a mortar and pestle, the alfalfa hay was ground into a fine powder. This was weighed out to 0.3 grams, using an electronic top-loading balance, and placed in an Erlenmeyer Flask.

### Ionic Liquid Pretreatment

1-butyl-3-methylimidazolium chloride, an ionic liquid, was used for the pretreatment of the alfalfa hay. Three grams of the ionic liquid were weighed, using the electronic top-loading balance. This was then placed in the Erlenmeyer flask containing the ground up biomass. The ratio of Ionic Liquid to biomass, alfalfa hay, was 1:3 for each sample. The samples were then placed in crystallized dishes, filled with mineral oil, and heated at 80 degrees Celsius for either three, six, or nine hours.

### Acid Hydrolysis

After the pretreatment, the samples were removed from the hot plate and allowed to cool. Ten milliliters of 0.5 M Hydrochloric acid was then added to each sample. The samples were placed back in the crystallized dishes containing the mineral oil. The samples were then heated at increments of either 3, 6, or 9 hours at 80 degrees Celsius. After they were heated, the samples were allowed to cool. After 30 minutes, 10 mL of 0.5 M Sodium Hydroxide was added to each flask in order to neutralize the contents of the flask.

### Centrifugation

The contents of the flask were transferred into centrifuge tubes. The samples were then centrifuged for two rounds. In the first round, the samples were spun at 2010 rpm for 10 minutes. In the second round, the samples were spun at 2500 rpm for five minutes. This process helps separate the plant mass from the liquid portion.

### Filtration

A filtration system was set up, which involved breaking glass pipettes at the elongated section. Each pipette was then filled with glass wool fiber, sand, and charcoal. The glass wool fiber was inserted first, followed by the sand, and then charcoal. A thin layer of sand was added to the charcoal. Charcoal is essential, because it filters out the color of the sample from brown to clear. The liquid from the centrifuged samples was then pipetted into each filter. The filtration set-up was repeated until all the liquid was filtered, and came out completely clear.

Figure 2. Ground Alfalfa Hay with Ionic Liquid



Figure 4. Filtration Set Up



Figure 3. Acid Hydrolysis of Ground Alfalfa Hay and Ionic Liquid

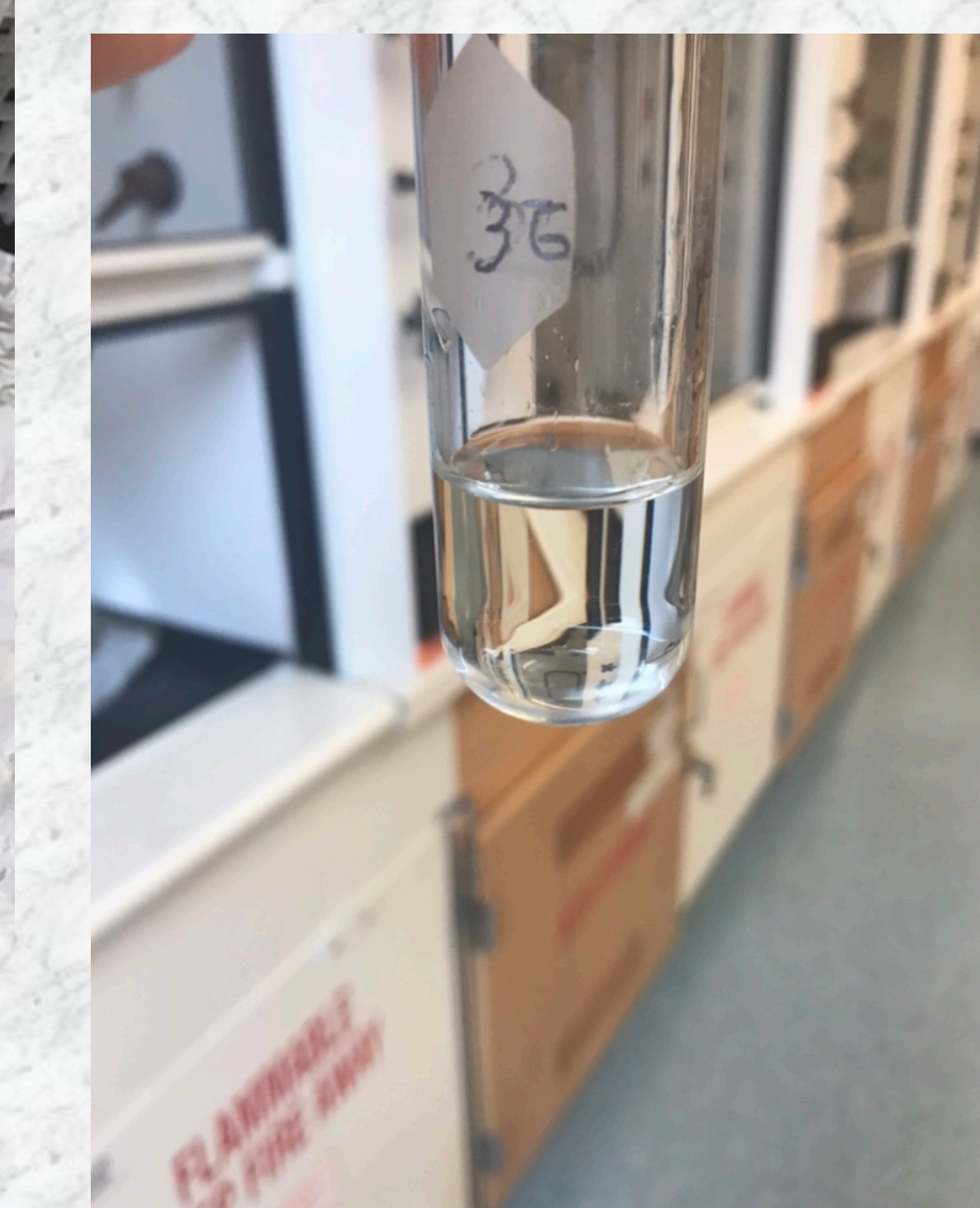


Figure 5. Clear filtered sample

## Further Directions

Future work could involve trying new methods of filtration, which would take less time removing the color of the samples after being centrifuged. A new approach could include increasing the amount of charcoal in the filters, because of its effectiveness in filtering the dark color from the samples. Future work also includes the testing of the samples, using the Dinitrosalicylic acid reagent test, in order to determine the glucose yield. The glucose yield of these samples can then be compared to the glucose quantification of other biomasses, such as rice husks or sawdust.

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