Deep Eutectic Solvents: Effect of Pre-treatment of Biomass to Enzymatic Digestion

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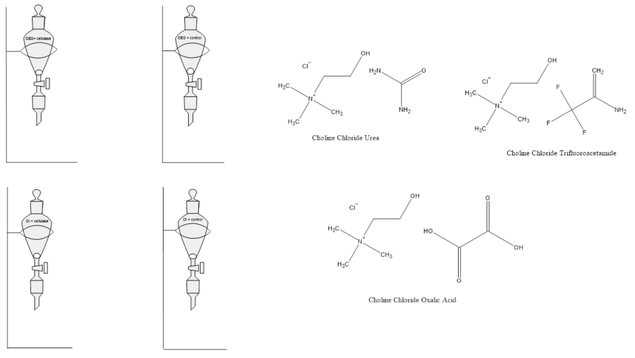
Abstract

As the demand for greener fuel sources increases, renewable fuel sources are being studied. Biofuels obtained from cellulosic sugars in biomass sources are one such alternative fuel source. Due to the properties of cellulose and lignin in biomass, pre-treatment methods need to be conducted to assess the enzymatic access to cellulose. This study used deep eutectic solvents (DES) as a pre-treatment strategy to weaken the intermolecular forces between cellulose and lignin. DESs are prepared by mixing a solid state hydrogen bond donor and a solid state hydrogen bond acceptor yielding a liquid. Three DESs were synthesized with choline chloride serving as the hydrogen bond acceptor for each and urea, trifluoroacetamide, and oxalic acid serving as hydrogen bond donors. The biomass source, corn stover from *Zea mays*, was then incubated with each DES. Of the three synthesized DESs, the urea-choline chloride DES produced the best results when incubated with cellulase enzyme. Since the urea-choline chloride DES produced the most significant deconstruction, the DES was recycled and reused three more times to test its ability for reuse in subsequent incubations with fresh biomass.

Background

Biofuels are produced through the process of breaking cellulosic sugars into ethanol. These cellulosic sugars are found as lignocellulosic biomasses.[1,2] Deep eutectic solvents (DESs) are composed of a hydrogen bond donor and a hydrogen bond acceptor and have been found to be able to weaken the bonds between lignin and cellulose.[3]
This project synthesized three different deep eutectic solvents and tested their ability to liberate glucose from cellulose after weaking the intermolecular force between cellulose and lignin.

Figure 1. Setup for Separatory Funnel System and Structures of DESs



Materials and Methods

- A glucose standard curve was generated using known concentrations of glucose and 3,5-dinitrosalicylate
- Sweet corn stover, spp. *Zea mays*, obtained from TN Tech Agriculture department chopped using a hand chopper
- Deep eutectic solvents were prepared by mixing the hydrogen bond donor and the hydrogen bond acceptor in a 2:1 mole ratio
- The chopped corn stover was incubated with the deep eutectic solvent for 24 hours in a separatory funnel seen in figure 1
- The corn stover was then incubated with 0.05 M citrate buffer and cellulase enzyme from *Aspergillus niger* for 24 hours
- The DNS colorimetric assay was used to determine the amount of glucose liberated from cellulose via absorbance

Results and Discussion

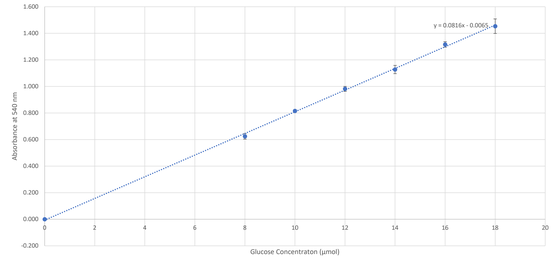
Three different deep eutectic solvents were prepared and tested with this assay. All of the deep eutectic solvents used choline chloride as the hydrogen bond acceptor while urea, oxalic acid, and trifluoroacetamide were all used as separate hydrogen bond donors.
After the full 48 hour incubation, only one of the prepared deep eutectic solvents, urea-choline chloride, was found to be able to allow access of cellulase enzyme to liberate glucose from cellulose via this method. Results from the incubation is shown in table I with each sample containing 1.0 mL of the urea-choline chloride DES.

Table I. Absorbance of Urea-Choline Chloride DES Samples

Sample ID	Absorbance	Glucose Conc. (µmol/mL)
1	0.410	5.10
2	0.100	1.31
3	Could not collect data	0.00
4	0.305	3.817
5	0.409	5.09
6	0.433	5.39

Using the equation obtained from the glucose standard curve shown in figure 2 and this absorbance data, the amount of the glucose liberated from cellulose could calculated. With all of the absorbances averaged together, 4.11 µmol of glucose was determined to have been liberated from the corn stover..

Figure 2. Glucose Standard Curve Graph



As deep eutectic solvents have been shown to be able to be recycled and reused, experiments were carried out to test how many times the deep eutectic solvent could be used and still be able to liberate glucose from cellulose. Since the urea-choline chloride DES was the only deep eutectic solvent which liberated glucose from cellulose, it was chosen to test its ability to be recycled and still liberate glucose. The same incubation procedure of the chopped corn stover was carried out with the deep eutectic solvent incubation followed by the citrate buffer and cellulase enzyme incubation.

The first reuse test did not achieve any measurable results but a second experiment with the same solvent did produce measurable results. The third use of the deep eutectic solvent was found to liberate glucose from cellulose which indicated the corn stover needed to be chopped reasonably close to the incubation date. The deep eutectic solvent was tested once more with corn stover and found to still be able to liberate glucose from cellulose after 4 uses.

While the urea-choline chloride DES was found to allow access of the cellulase enzyme to liberate glucose even after four uses, the amount of glucose liberated was found to decrease with each use. This could be expected as the amount of deep eutectic solvent did decrease with each subsequent incubation. The amount of glucose liberated was then calculated in grams and moles using the equations shown below.

$$Equation\ 1. \quad Abs = 0.0816C - 0.0065$$
$$C = (Abs + 0.0065)/0.0816$$

$$Equation\ 2. \quad C\ \frac{\mu mol}{mL}\ x\ \frac{1\ mol}{10^6\ \mu mol}\ x\ 1\ mL$$

Acknowledgements

Thanks to Dr. Jeffery Boles and Dr. Andreea Cojocaru for providing all the chemicals and equipment used for the duration of this research. Thanks to the Tennessee Tech Agriculture Department for providing the corn stover used in this research.

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