

Introduction and Research Rationale:

Biorefineries are facilities that convert plant biomass into fuels, power, heat and value-added chemicals. In terms of major unit operations (i.e. feedstock sourcing, cracking/depolymerization, organic synthesis, product purification) biorefining is analogous to petroleum refining. The main differences are that biorefineries use raw materials (plant feedstocks) which are both renewable and domestically abundant, and often utilize microorganisms as tiny synthetic chemists.

Two CMS teachers from the Charlotte Teachers Institute's 2018 Summer Research Experience Program and one high school student volunteer participated in an on-going research project at Queens University of Charlotte to make biofuels from switchgrass (*Panicum virgatum*), a perennial plant native to North America. Biofuels, such as ethanol, are produced from the fermentation of glucose by a microbe, such as yeast.



Figure 1: (left to right) Erika Williams, high school biology teacher; Carla Aaron-Lopez, middle school art teacher; Andrew DeWeese, high school student

The plant cell wall is depicted in Figure 2, showing principal polymeric components (cellulose, hemicellulose and lignin). Their monomers, comprising sugars and monolignols, differ between plant types (e.g. grasses, softwoods and hardwoods).

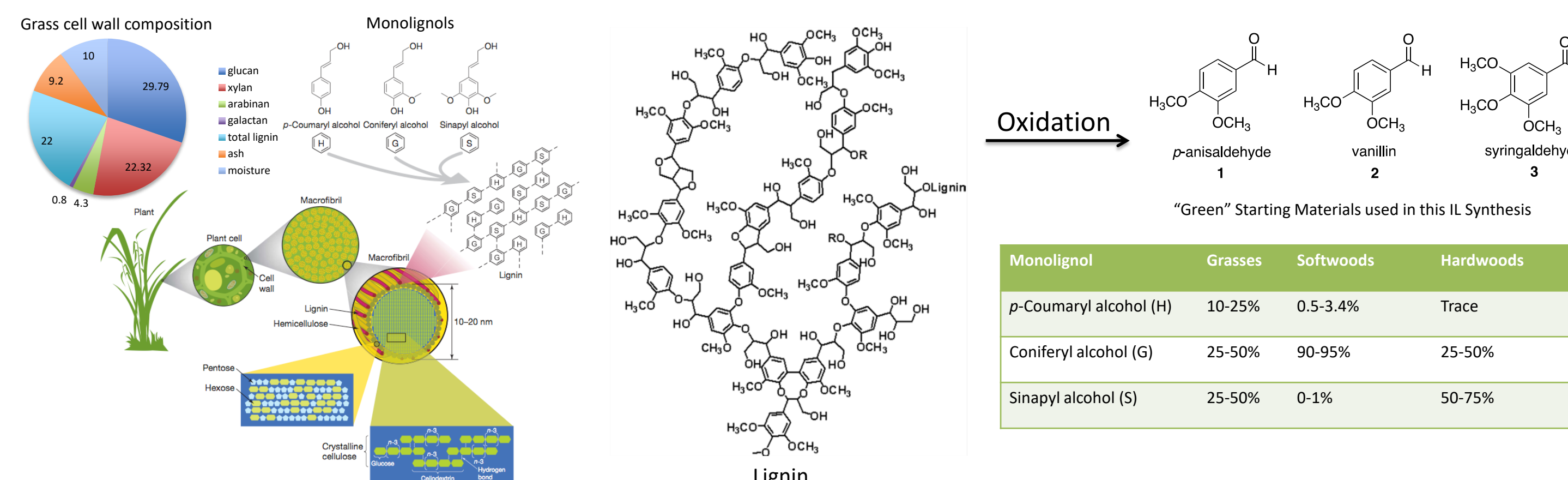


Figure 2: Left = Depiction of plant cell wall showing lignin, cellulose and hemicellulose (Image: Rubin, 2008). Inlay = Approximate compositional analysis of switchgrass used in this study. Middle = Representative structure of lignin macromolecule. Right = Benzaldehydes derived from lignin oxidation. Table = Monomeric lignin composition across plant types.

Our overall project goal was to conduct a series of biomass pretreatments in order to convert switchgrass into fermentable sugars (glucose and xylose). Additionally, since lignin is typically considered a low-value product of biorefining we sought to *utilize the lignin as a solvent for processing the sugars*. Lignin can be depolymerized by oxidation to give *p*-anisaldehyde, vanillin and syringaldehyde (Figure 2 top right).

Biomass pretreatment is designed to separate plant polymers so that the cellulose can be easily accessed by cellulase enzymes which hydrolyze the cellulose to glucose. Additionally, hemicellulose is hydrolyzed by hemicellulase enzymes to produce xylose.

We used a series of model compounds (benzaldehyde, *p*-anisaldehyde, vanillin and syringaldehyde) to represent oxidized lignin for the synthesis of “bio-based” ionic liquids (ILs). Certain ionic liquids are exceptional at dissolving both cellulose and lignin. A 2 x 2 study was then performed to compare pretreatment efficacies of bio-based vs. petroleum-based ionic liquids. The structures and images of the ILs tested appear in Figure 3.

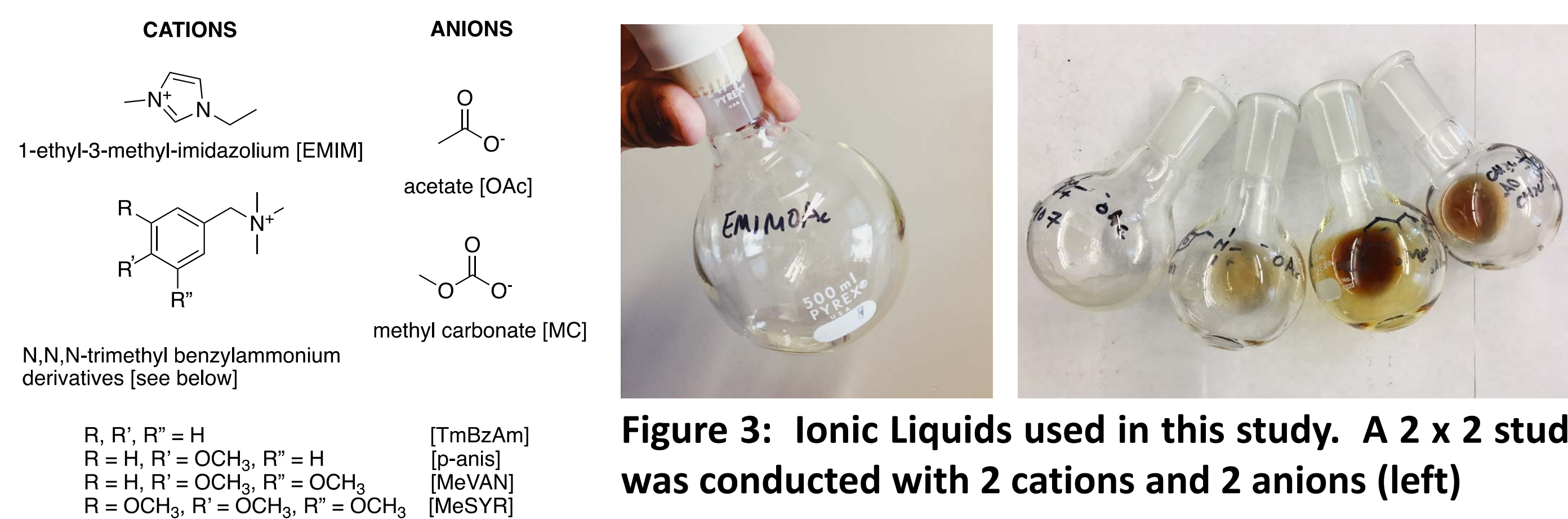
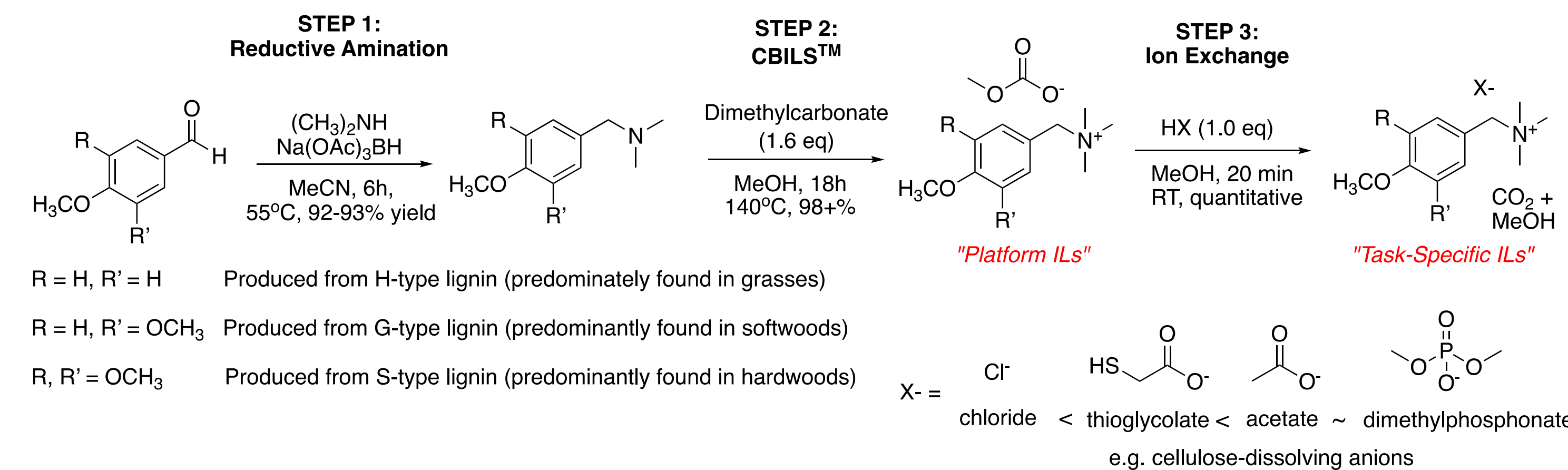


Figure 3: Ionic Liquids used in this study. A 2 x 2 study was conducted with 2 cations and 2 anions (left)

Methods for Switchgrass Pretreatments using Ionic Liquids:

Ionic Liquid Synthesis from Lignin Model Compounds:

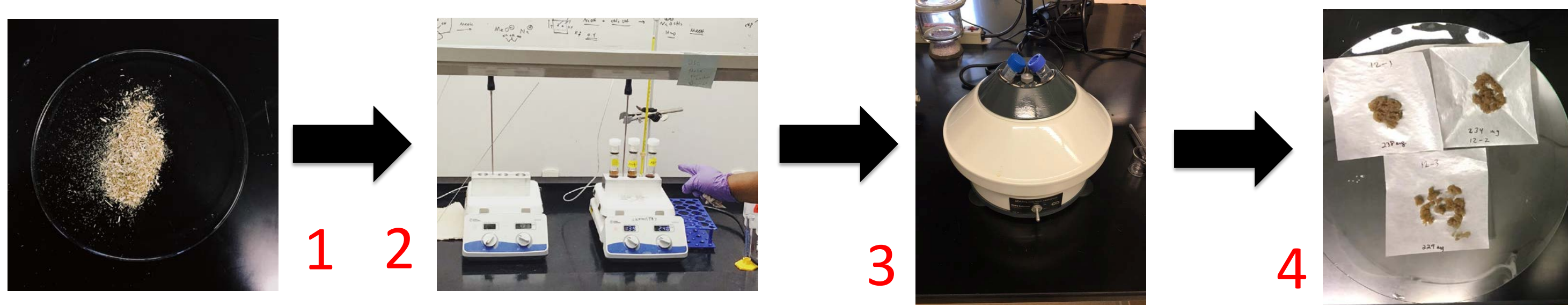
A typical synthesis started with reductive amination of 10-20 g of a lignin-derived benzaldehyde (**1-3**) using dimethylamine gas and sodium triceoxyborohydride in a 600 mL Parr pressure reactor (Scheme 1, Step 1). The products were purified using an acid/base extraction protocol¹ to give 95+% pure dimethylbenzylamines, monitored by GC-MS and TLC (2:1 hexane:EtOAc + 1% dihexylamine). Tertiary amine products were subjected to alkylation using dimethylcarbonate in the CBILS™ process² (Step 2). The reactions were monitored by HPLC and TLC (8:4:2 hexane:EtOAc:MeOH +1% dihexylamine). Workup involved removal of methanol solvent by rotary evaporation to give “Platform ILs” – trimethylbenzyl ammonium methyl carbonates. The methyl carbonate anion was easily exchanged with 1 molar equivalent of any acid with pKa < 9 (Step 3). We selected acetic acid as it is well-know to dissolve cellulose when paired to alkyl imidazolium cations.



Scheme 1: Synthesis of ILs from benzaldehyde and lignin model compounds (1-3)

Pretreatment Experiments:

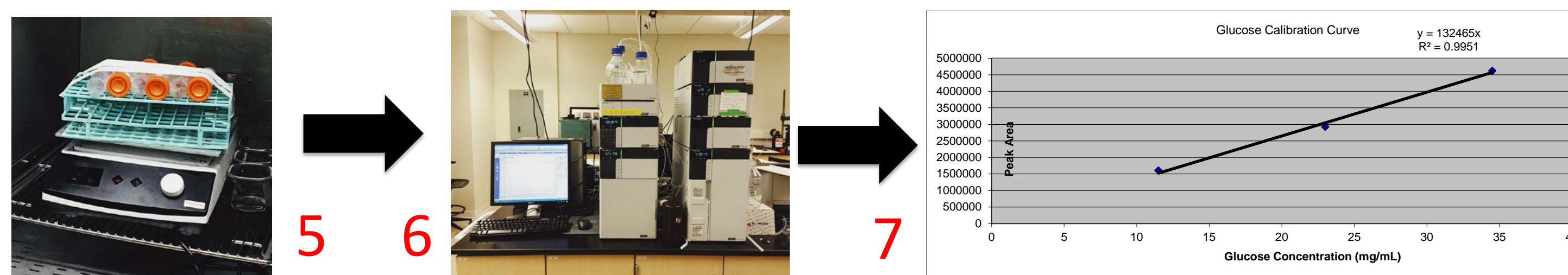
With the ILs in hand, it was time to conduct the pretreatment experiments (Figure 4, Experiments 1-5). Approximately 2 g of IL was added to ~ 100 mg of switchgrass (**1**) (5% solids loading) and heated in #15 Ace Glass Pressure Tubes on a thermocoupled aluminum block for 2-3 hours at 140°C (**2**). Upon completion, the mixture was washed with 1 x 10 mL methanol and 4 x 10 mL of DI water (60°C) to precipitate IL-free cellulose pulp (**3**) (4500 RPM, RT). Depending on IL and pretreatment severity approximately 20-50% of the biomass was recovered from the original 100 mg of switchgrass after drying overnight at 65°C in oven (**4**). Interestingly, the 1st wash (containing most of the IL) from TMBzAM MC pretreatments was readily converted to its acetate suggesting that ion exchange with a hydrophobic anion would serve to recycle MC ILs after pretreatment.



Enzymatic Saccharification and HPLC Quantification of Glucose and Xylose:

Washed biomass was pH adjusted to 5.2 using 0.9 mL of citric acid/citrate buffer (= 10% solids loading – calculated from original 100 mg switchgrass). An enzyme “cocktail” containing 155 mg cellulase (CTEC) and 25 mg hemicellulase (HTEC) was prepared in 2.85 mL citrate buffer (total cocktail volume) and aliquots were added to hydrolyze the cellulose and hemicellulose to fermentable sugars, glucose and xylose. Saccharification reactions received approximately 17 µL of cocktail, corresponding to 0.92 mg cellulase and 0.15 mg hemicellulase. This is a reasonable enzyme loading of 31 mg CTEC/g glucan and 6.7 mg HTEC/ gram xylan.

Reactions were allowed to incubate at 50°C for 15 or 45 hr with gentle mixing (80 RPM) (**5**). Sugars were subsequently quantified on the HPLC (Biorad Aminex 87-P Column 300 x 7.8 mm, 100% water mobile phase, RI detection) by filtering sample (0.2 micron) and direct injection onto HPLC (**6**) (i.e. no dilution needed). Glucose yields were quantified by comparing peak areas to a calibration curve prepared from authentic standards at 11.5, 23 and 34.5 mg/mL (**7**).



Results and Conclusions:

Additional pretreatment experiments (6-8b) were performed with Avicell PH 101 microcrystalline cellulose. While the enzyme loading was doubled, the hydrolysis reaction time was lowered by a factor of 3 and pretreatment temperature and time was lowered to 90°C and 30 min, respectively. Swelling of Avicell was apparent within minutes of addition of TMBzAM OAc at all temperatures tested. After a shortened enzymatic hydrolysis, with ~ 2x the enzyme loading, glucose yields were measured as high as 42 ± 12% (Exp. 8). It is noteworthy that the lower temperature Avicell pretreatment gave the best glucose yields (Exps. 6-8).

Exp. number	Feedstock	Enzyme Loading (mg/g glucan)	Enz Rxn Time (hr at 50°C)	Pretreatment Conditions
1	Putnum Switchgrass	30 mg cellulase : 4.8 mg hemicellulase	45	140C_3hr_Water
2	Putnum Switchgrass	30 mg cellulase : 4.8 mg hemicellulase	45	140C_2hr_TMBzAM MC
3	Putnum Switchgrass	30 mg cellulase : 4.8 mg hemicellulase	45	140C_2hr_TMBzAM OAc
4	Putnum Switchgrass	30 mg cellulase : 4.8 mg hemicellulase	45	140C_3hr_10% NaOH
5	Putnum Switchgrass	30 mg cellulase : 4.8 mg hemicellulase	45	140C_3hr_EMIM OAc
6	Avicel PH 101	65 mg cellulase : 10 mg hemicellulase	15	140C_30min_TMBzAM OAc
6b	Avicel PH 101	66 mg cellulase : 10 mg hemicellulase	15	140C_30min_TMBzAM OAc
7	Avicel PH 101	67 mg cellulase : 10 mg hemicellulase	15	120C_30min_TMBzAM OAc
8	Avicel PH 101	68 mg cellulase : 10 mg hemicellulase	15	90C_30min_TMBzAM OAc
8b	Avicel PH 101	69 mg cellulase : 10 mg hemicellulase	15	90C_30min_TMBzAM OAc

Table 1: Pretreatment and enzymatic hydrolysis reactions performed in this study

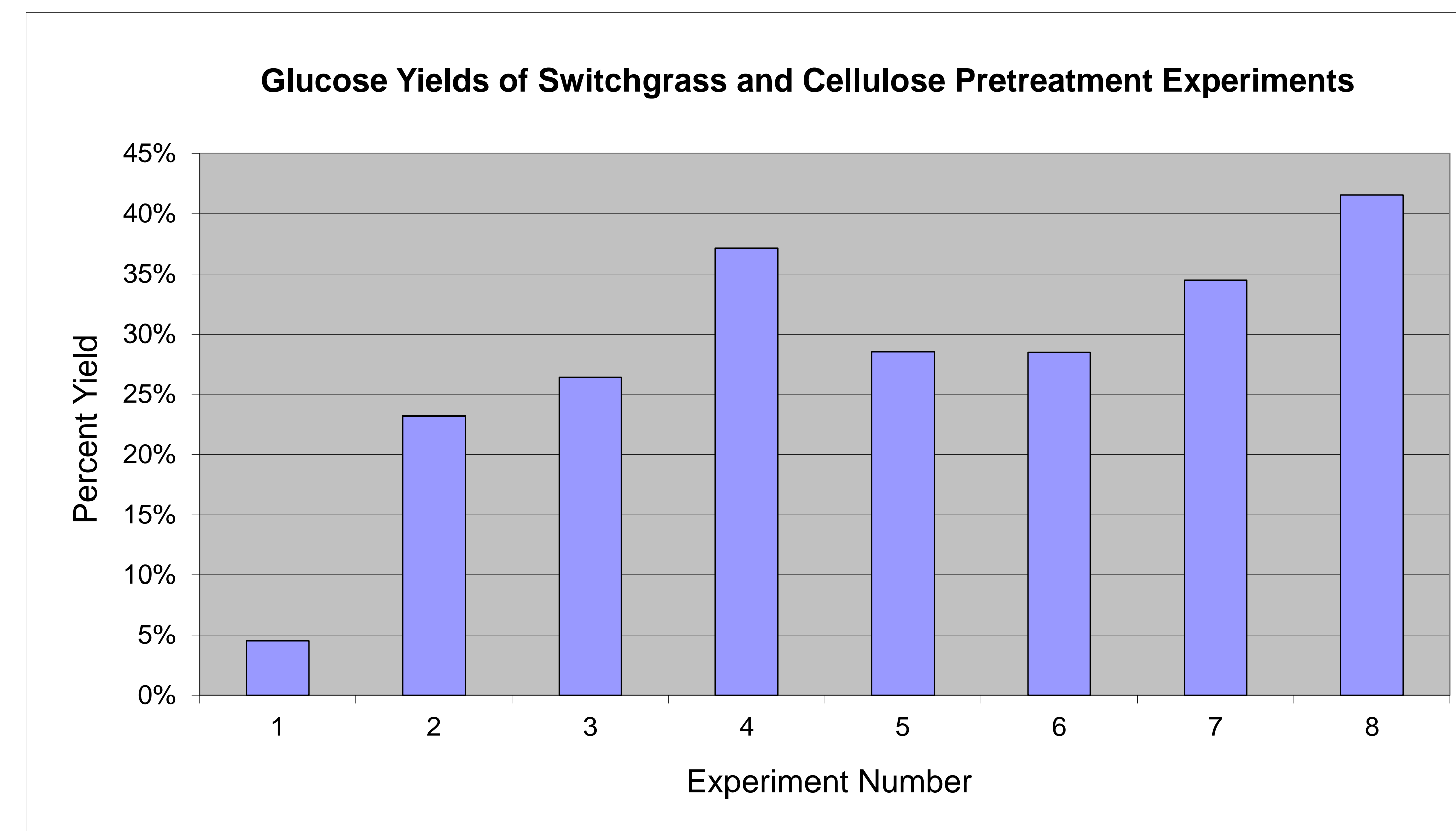


Figure 4: Glucose yields from pretreatment experiments shown in Table 1. Columns 6 and 8 represent average yields of duplicate experiments with standard deviations of ± 1% and 12%, respectively.

Conclusions:

A robust and efficient synthesis of a series of quaternary ammonium platform ionic liquids was obtained using dimethyl carbonate, an environmentally benign alkylating agent, and a series of benzaldehyde starting materials derived from lignin across all species of terrestrial plants. A 2 x 2 comparison of the newly prepared cations with 1-ethyl-3-methyl imidazolium using coordinating methyl carbonate and acetate anions was performed. Results showed that glucose yields from TMBzAM OAc (Exp 3, 26%) were similar to those of EMIM OAc (Exp 5, 29%) under similar pretreatment and enzyme hydrolysis conditions. Also noteworthy is that EMIM MC pretreatment was run as a 30% solution in methanol and gave sugar yields <5 %. This experiment could be repeated as a neat EMIM MC IL to accurately complete the 2 x 2 comparison. Our results from the model TMBzAm cation suggests that the lignin-derived cations will perform similarly, as they only differ in 1-3 methoxy groups (Figure 3). Future work will be directed towards repeating switchgrass and Avicell pretreatments with the lignin-derived ILs.

Works Cited:

- ¹Socha AM, et al. (2014). *Efficient biomass pretreatment using ionic liquids derived from lignin and hemicellulose*. *Proc. Nat. Acad. Sci* Vol. 111, No. 35, pp E3587–E3595
- ²Kalb R, et al. *Carbonate Based Ionic Liquid Synthesis CBILS™: Thermodynamic Analysis*. *Phys. Chem.Chem. Phys.* 2016, 18, 31904

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