Lignocellulosic Biomass Pulping Lab:
An Introduction to Pretreatment

NSF ATE Project 1601636
Chemical and BioEnergy for Sustainability

Introduction:

Plants are autotrophs, organisms that produce organic molecules by converting light energy into chemical energy in the process of photosynthesis. During photosynthesis, carbon dioxide (CO_2) and water (H_2O) are converted into molecular oxygen (O_2), sugars, lipids and amino acids through a series of enzyme-catalyzed reactions. Glucose ($C_6H_{12}O_6$) is the major product of photosynthesis, and used by higher organisms for cellular respiration. The latter process yields energy (2880 kJ/mol of glucose) and CO_2 and is similar to a fuel combustion reaction (Figure 1).

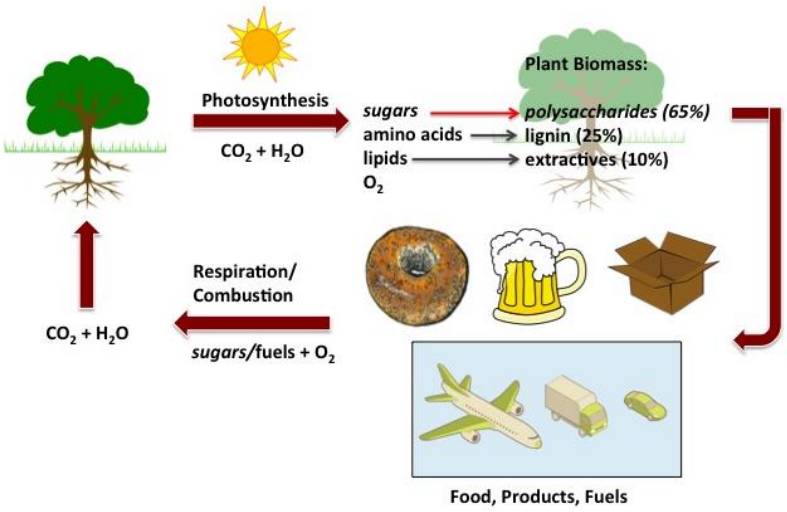


Figure 1: A cartoon representation of the complex processes of photosynthesis and cellular respiration/combustion. Plant-derived polysaccharides, such as cellulose and starches, are central to both processes, making biofuels "carbon neutral".

Plant biomass contains the two most abundant *biopolymers* on earth – *cellulose* and *lignin*. Cellulose comprises approximately 45% of the dry plant mass and is formed by the "upside-down" *polymerization* of glucose. *Starches* are mostly found in the fruit of plants, and are formed from the "right-side-up" polymerization of glucose (Figure 2). *Hemicellulose* comprises approximately 20% of the dry plant mass, and is formed from the polymerization of glucose with other sugars, such as xylose and mannose. Collectively cellulose and hemicellulose are known as *polysaccharides* or *carbohydrates*.

Certain microorganisms such as yeast and *E. coli* can be engineered to *ferment* carbohydrates into beverages, foods and fuels. Cellulose is also used to make paper

and paper products, such as cardboard. The digital economy is causing declining paper production in the United States, while developing countries such as China are increasing paper production. Moreover, increasing reliance on product shipments (e.g. Amazon), are providing a steady demand for cardboard products worldwide.

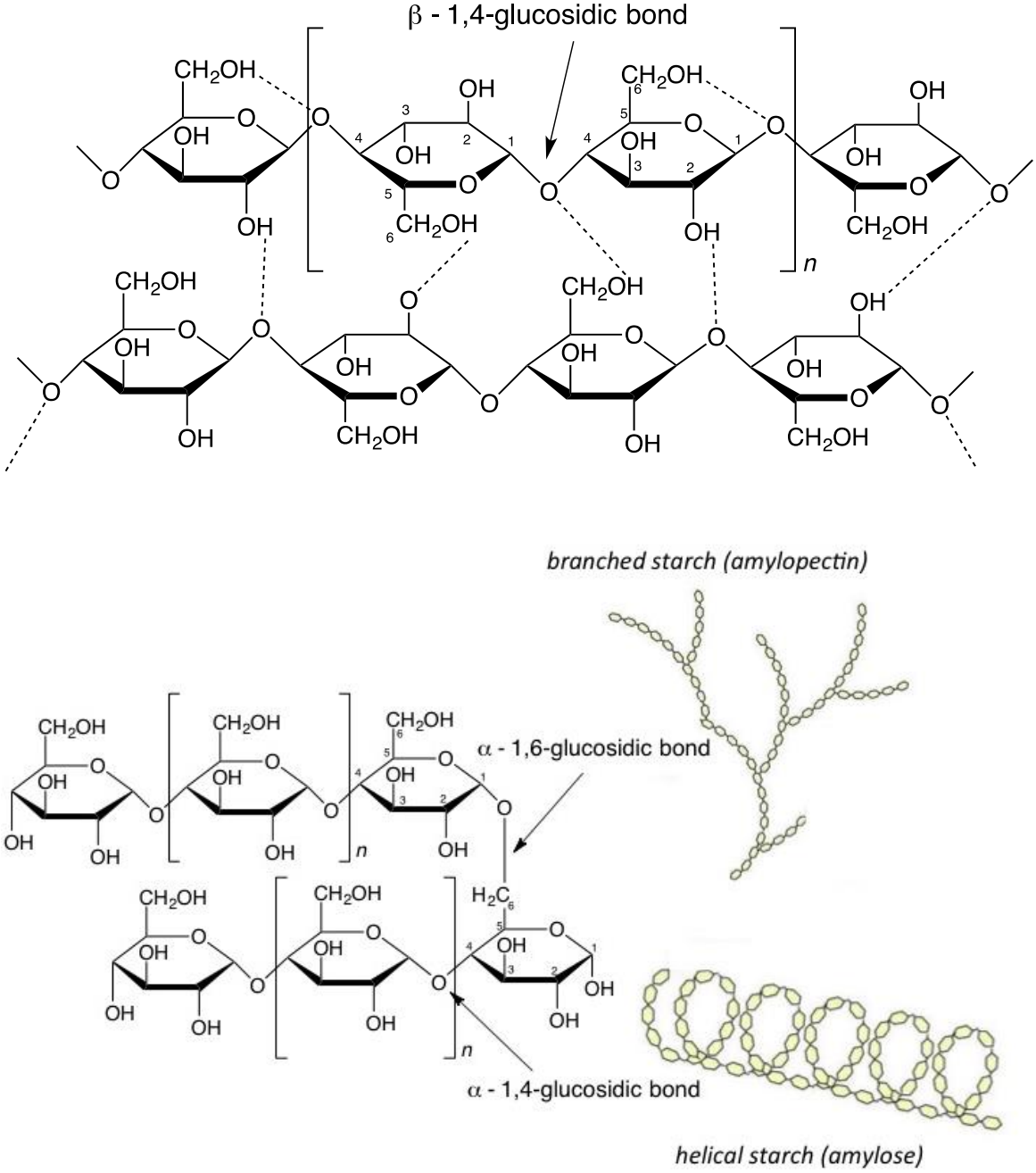


Figure 2: (Top) Cellulose containing the characteristic β-1,4 glucosidic linkage and inter- and intra-molecular H-bonds. The bracketed repeating unit of cellulose is called *cellobiose*. (Bottom) The α-1,4- and α-1,6- glucosidic bonds of "complex carbohydrates" (a.k.a. starches) and their corresponding branched and helical macromolecular structures.

Note - Different plant types contain different percentages of individual sugar monomer types and thus hemicelluloses. For example, grasses and hardwoods softwoods contain mostly xylose, and softwoods contain mostly mannose, in their hemicelluloses.

Lignin is a *heterogeneous* polymer that is biosynthesized from the amino acids phenylalanine and tyrosine during developmental growth, and during stress events such as wounding and pathogen infection, of a plant. It is generally accepted that the first occurrence of lignin began when land plants evolved from algae because the newly inhabited terrestrial environment required upright growth, and the rigid aromatic polymer provided structural support (Vanholm, 2010). It is noteworthy, however, that lignin has been found in some marine species of red algae, and is thought to have protected these plants from the force of breaking waves (Martone, 2009).

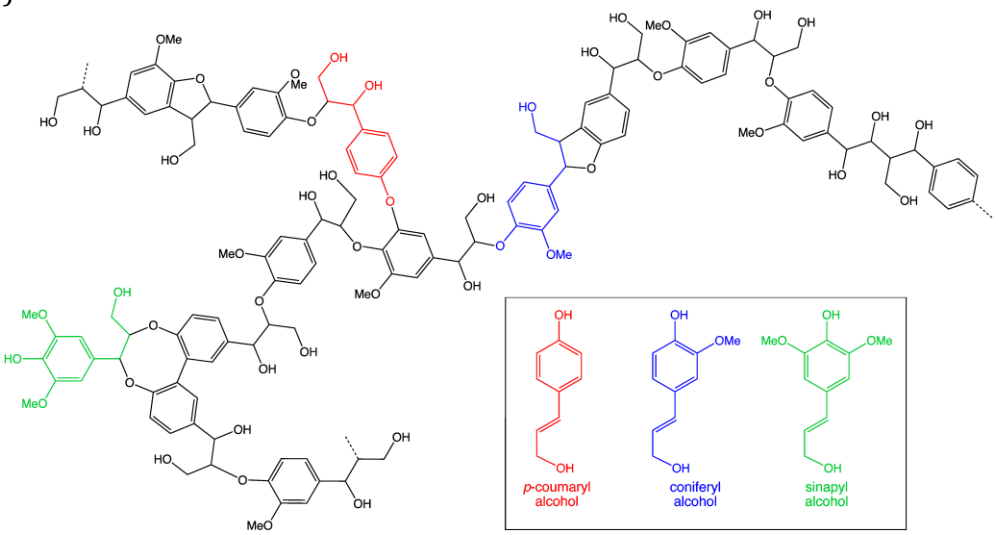


Figure 3: The general structure of the lignin polymer showing its monomeric units: *para*-coumaryl alcohol (H-lignin), coniferyl alcohol (G-lignin) and sinapyl alcohol (S-lignin).

Lignin comprises approximately 25% of the dry plant mass, and its heterogeneity is largely due to two factors. First, different types of plants use different monomeric units (*monolignols*) to build their lignins. Softwood gymnosperms (e.g. pine, fir, spruce, cedar, juniper, redwood) have mostly G-type (guaiacyl) lignin, derived from the monomeric unit coniferyl alcohol (Figure 3). Hardwood angiosperms (e.g. beech, hickory, mahogany, maple, oak, teak, walnut) use both G-type and S-type (syringyl) monomeric units, derived from coniferyl and sinapyl alcohols, respectively. Angiosperm grasses (e.g. corn, wheat, rice, oats, barley, millet, bamboo, switchgrass, sorghum, sugarcane) incorporate *p*-coumaryl alcohol units into lignin. Table 1 summarizes lignin monomer composition among the three different plant types. The syringyl/guiaicyl ratio or "S/G ratio" between different bioenergy crops varies significantly, and can determine their utility as biofuel feedstocks.

Table 1: The compositional ratio of monolignols in higher plants.

Lignin Monomer	Softwood	Hardwood	Grasses
p-coumaryl alcohol	0.5-3.5%	Trace amounts	10-25%
coniferyl alcohol (G)	90-95%	25-50%	25-50%
sinapyl alcohol (S)	0-1%	50-75%	25-50%

The most significant factor contributing to the structural complexity of lignin is the diversity in chemical linkages between the individual monomeric units. There are at least 16 different linkage types (Figure 4) with the most common linkages being the β-O-4 (45-50%), the 5-5 (18-25%) and the β-5 (9-12%). As you may expect, each of the different bonding patterns requires different energy levels (kJ/mol) to break, and the energy levels can be lowered by using catalysts.

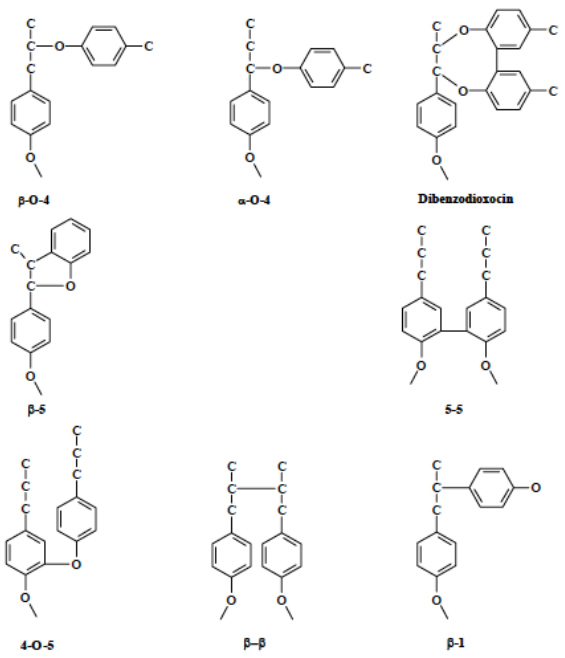
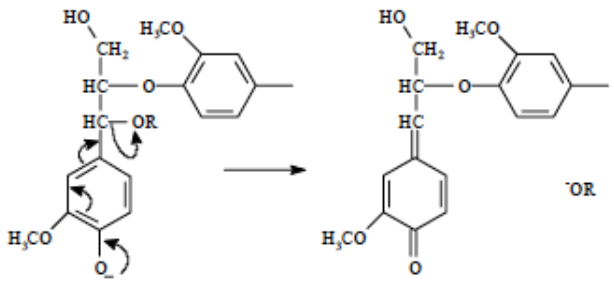


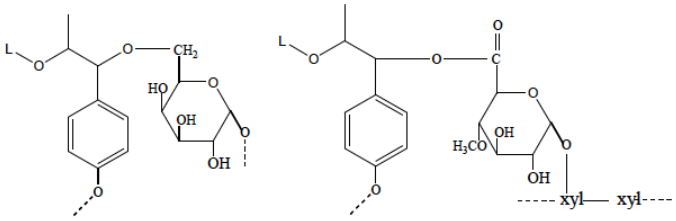
Figure 4: Several common inter-unit linkages found in the lignin macromolecule.

Lignin is generally considered as a by-product of the paper and biofuel industries, but is gaining more recognition as a low cost starting material for chemistry and materials science. There are many uses for lignin uses ranging from low to high value. For example, lignin is used as a dust-control agent on roads in developing countries, to improve properties of cement and asphalt, to make foams, plastics, polymers, wood products and carbon fibers. Additionally, lignin is used as an antioxidant in cosmetics, as a precursor to high value chemicals such as *resorcinols, catechols, quinones* and *vanillin*. Lignin is also used in certain types of batteries, and can be converted into fuels, greases, agricultural additives, dye dispersants and burned for heat. In this experiment, you will devise a method to separate the primary components of biomass: extractives, cellulose and lignin.

Although biological or physical processes cannot easily separate the cellulose and lignin polymers, a chemical process known as *pulping* can be employed. There are many variations on pulping, and the *soda pulping* process, invented in 1851 by Burgess and Watts, uses dilute sodium hydroxide (NaOH) to cleave *α-aryl ether bonds*. This bond cleavage serves two purposes. First, if the R group in Figure 6 is another component of lignin, smaller (more soluble) lignin fragments are generated.

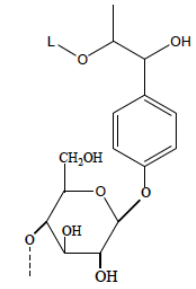
Next, if R represents cellulose and/or hemicellulose (Lawoko, 2005), soda pulping can be effective for separating lignin from these carbohydrates.

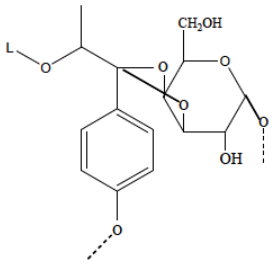




Benzylether LC-bond

Benzylester LC-bond





Phenylglycoside LC-bond

Acetal type LC-bond

Figure 6: (Top) The cleavage of α-aryl ether bonds using the hydroxide ion.

(Bottom) examples of lignin-carbohydrate linkages.

Part 1: Soxlet Extraction of Biomass Feedstocks

"Extractives" are an undefined set of compounds that differ depending on the plant species and the solvent used for extraction. Extractives can include waxes/oils, terpenes, phenolics, coumarins, flavonoids, etc. For example, extractives reported from *cooperage* oak include ellagitannins, whiskey-lactones, eugenol and vanillin (Doussot, 2002). Because they are typically a low percentage of the overall biomass composition, and of higher value than biopolymers (e.g. cellulose, hemicellulose, lignin) extractives are typically removed prior to pretreatment using solvent extraction. In our experiment, we will use an apparatus known as a soxlet (Figure 5) to perform this extraction.

Overall Objectives:

To gain an understanding of the process of extracting natural products from biomass and translate these products to a biorefining process using knowledge of product yield, composition and solvents required for extraction.

Equipment and Materials Needed:

Three types of biomass (hardwood, e.g. oak; softwood e.g. pine; grass, e.g. switchgrass)

Soxlet extractor

Gauze or glass wool

Round bottom flasks

Non-polar solvent (hexane, ether, or ethyl acetate)

Polar solvent (methanol or ethanol)

Rotary evaporator or another type of solvent recovery system

Prelab Questions:

You will perform a soxlet extraction on biomass using two solvents of increasing polarity:

1) Why do you first perform extraction with the non-polar?

2) What compounds do you expect to extract from feedstocks such as pine, oak, eucalyptus, grasses?

3) What is the approximate yield and value of these compounds as compared to biofuel?

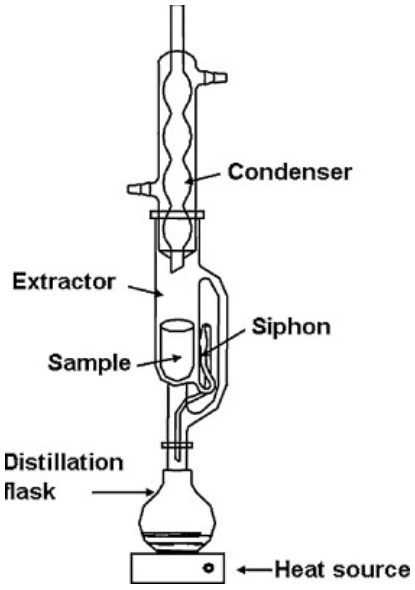


Figure 5: Soxlet setup for performing extractions on biomass.

Procedure:

Pack a known amount* of biomass (sample) into the soxlet (extractor) with gauze or glass wool to prevent biomass from passing into the distillation flask.

Perform the non-polar solvent extraction with a known amount of solvent for 1 hr.

Perform the polar solvent extraction with a known amount of solvent for 1 hr.

Dry your extracts on the rotary evaporator to obtain an accurate mass of extractives.

Perform GC-MS on extractives. Can you identify any compounds and their relative yields?

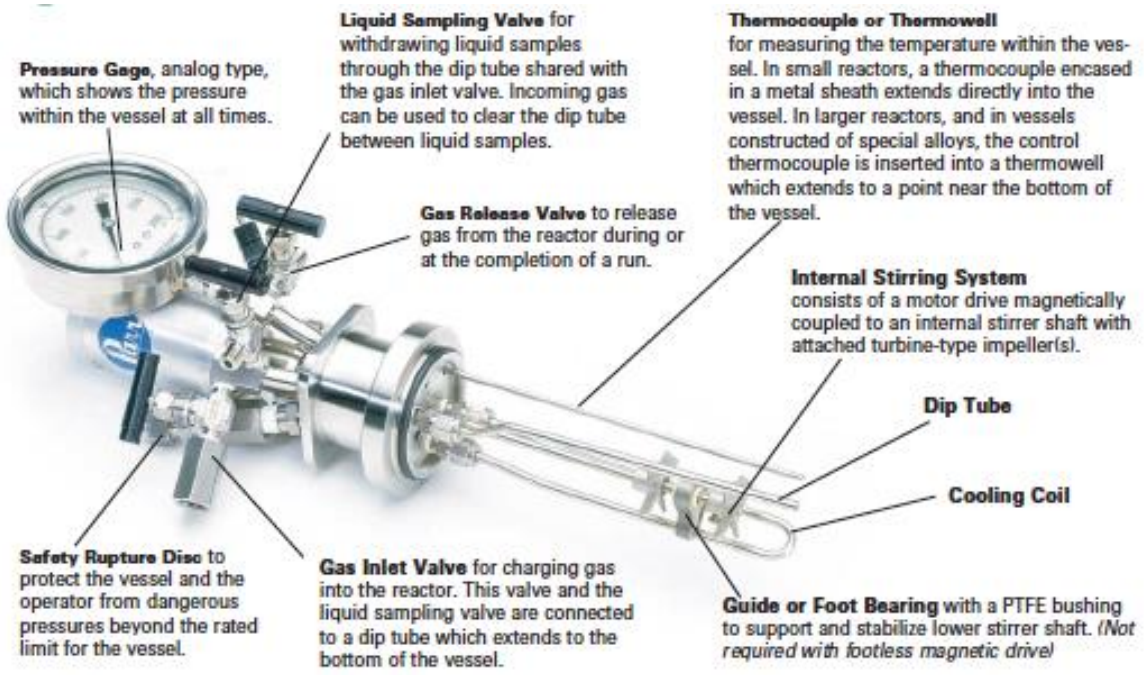
** note that the type and amount of biomass and solvents used in this experiment are dependent on availability and size of soxlet apparatus used.*

Part 2: Biomass Pretreatment in a Pressure Reactor using the Soda Pulping Method

Pulping reactions typically occur in a reactor called a *digester*. In the case of this lab, the digester will be a Parr pressure reactor. The reactor is capable of reaching temperatures of 300 degrees Celsius and pressures of 2000 psi! The reactor was built from a nickel alloy called Monel, and is very resistant to alkaline corrosion – even at elevated temperatures and pressures.

Figure 7: (Top) A 75 mL Parr pressure reactor complete with heating mantle, overhead stirring and control unit.

(Bottom) the inner parts of the reactor vessel.



Overall Objectives:

To begin to understand biopolymer separation procedures using thermochemical methods, and to perform oxidative and inert methods to separate cellulose from lignin.

Pre-lab Questions:

1. Define all of the *italicized words* found in the Introduction.
2. Based on your selected feedstock, what percentages of the biomass as cellulose pulp and lignin do you expect to have at the end of the soda pulping process?
3. What is meant by a "mass balance" and what components will you include in a mass balance of the soda pulping process?

Key concepts/techniques:

- Understand the chemical differences between grasses, softwoods and hardwoods
- Develop a simple technique to determine the moisture content of biomass
- Understand the chemistry of a soxlet extraction
- Understand the chemistry of soda and Kraft pulping processes
- Clean, assemble, pressurize and inspect Parr Reactors for leaks
- Build a reaction program in SpecView to run Parr reactor
- Transfer data and plot reaction parameters (temperature, time, pressure, stir rate)
- Calibrate and measure reaction mixture samples with a pH meter
- Learn methods of centrifugation and rapid product separation and washing
- Understand chemical/sample waste vs. non-waste and procedures for handling
- Perform compositional analysis and mass balance on biomass samples

Equipment and Materials:

Parr pressure reactor (1)
Benchtop centrifuge (1)
pH meter (1)
Drying oven (1)
50 mL conical centrifuge tubes (12 per lab group)
500 mL beaker (1 per lab group)
8" watchglasses (2 per group)
stirplate and stir bars (1 per lab group)
25.0 grams of biomass (per lab group)
312.5 mL of methanol (per lab group)
250 mL of 0.5 M NaOH (per lab group)
25 mL of 6M HCl (per lab group)
Squirt bottle of DI water (1 per lab group)

Procedure:

The following steps are meant as a ***guide*** to help you develop your own, repeatable procedure for the compositional analysis/mass balance of a selected biomass feedstock. You will need to repeat your work *three times* to obtain data suitable for statistical analysis. You will then compare your data to that obtained by other members of the class.

Experimental Variables (to be determined by your instructor):

1) Reaction time – 30 min, 90 min, 120 min – Does reaction time affect yields and/or quality of lignin and/or cellulose?
2) Gas charge pressure (0 or 100 psi) – Does gas charge pressure affect yields and/or quality of lignin and/or cellulose?
3) Charge gas composition (O_2, N_2, H_2) – Does charge gas composition affect lignin yields and/or lignin molecular weights?
4) Biomass type (softwood, hardwood or grass). Do you observe a difference in cellulose yields and/or monolignol composition?

Weigh 25.0 g of biomass feedstock (e.g. willow oak *Quercus phellos* woodchips, approx. 1 cm x 1 cm x 1 mm).

Develop a method to determine the "moisture content" of biomass and plot your results. Hint - use approximately 2.5 g biomass by heating in an oven at 65^{o}C for at least 3 hr.

Dry biomass in oven at 65^{o}C overnight.

Determine moisture content again.

Add dry, extractive-free biomass to Parr reactor with 250 mL of 0.5M NaOH

Charge reactor with suitable gas. In order to perform Lignin Chromatography Lab (Lab 6 in this course), you will need to perform reactions under both inert (N_2) and oxidative (O_2) conditions.

Heat reaction to 170^{o}C for 1 hr. The amount of time that it takes to reach target temperature +/- 5^{o}C is not included in the 1 hr. Pressure should be approx. 90-100 psi. Set stirring to 415 RPM.

Allow to cool to 50^{o}C. Rinse reactor impellor with water to remove any solids/liquids and reach a total volume of 350 mL of combined material.

Cool reaction mixture to 4^{o}C in an ice bath. Centrifuge (4150 RPM, 5 min, 4^{o}C) to remove cellulose pulp. Combine cellulose pulp and wash 5x with water (100 mL each wash). Discard water washes into sink.

Record wet mass of cellulose pulp.

Dry cellulose pulp and/or determine moisture content of cellulose pulp.

Determine recovered mass of cellulose.

Calibrate pH meter and measure pH of reaction mixture. It should be ~ 13.0

Add 2mL aliquots of 6M HCl until pH reaches 2.0. Record volume of HCl used. Plot the pH change vs. volume of 6M HCl used.

Lignin will precipitate and must be concentrated via centrifuge (4150 RPM, 15 min, 4°C). Combine lignin pellets and wash with 5 x 100 mL. This water is acidic and cannot be poured down the sink.

Record wet mass of lignin.

Determine moisture content of lignin.

Determine recovered mass of lignin.

Complete your mass balance by estimating amount of *hydrolyzed* hemicellulose.

Where is this hemicellulose?

Product Analysis (to be determined by Instructor):

Analyze lignin by HPLC using DAD detector and size exclusion column (Lab 6)

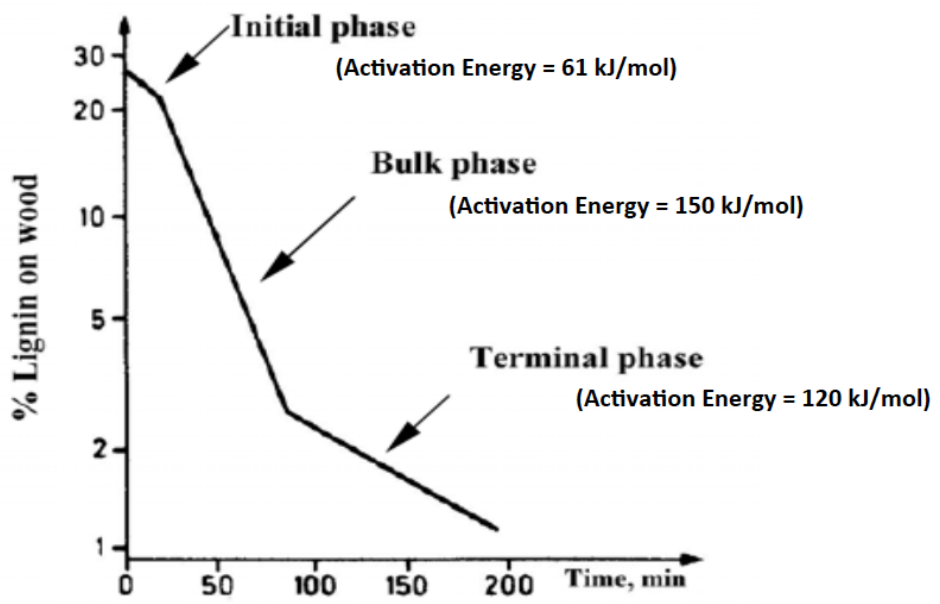
Perform IR and/or NMR on your lignin and cellulose pulp fractions.

Lab Report:

Why are biofuels considered "carbon neutral"?

Diagram a mass flow for the materials in your biomass. Do the masses of extractives, carbohydrates and lignin, agree with literature value for your feedstock?

How did the experimental variables affect your results? Plot your data. How does your data compare to the figure below? What are the bond energies for the most common lignin linkages α-O-4 and β-O-4? What are the effects of catalysts?



What was the experimental error in your measurements and how did they compare to the class average?

References:

1. Vanholme, R. et al. Lignin biosynthesis and structure. *Plant Physiology*. **2010**. 153, 3, 895-905. http://www.plantphysiol.org/content/153/3/895.full

2. Martone PT et al. Discovery of lignin in seaweed reveals convergent evolution of cell wall architecture. *Curr Biol* **2009**, 19, 169-175.

3. Doussot, F. et al Extractives content in cooperage oak wood during natural seasoning and toasting; influence of tree species, geographic location, and single-tree effect. *J Agric Food Chem*. **2002** 50, 21, 5955-61.

Helpful Links for Lab Report:

http://www.csebcc.org/Courses/Biofuels_GreenChem/CHM_31/Model_Lab_Format.pdf

http://biorefinery.utk.edu/technical_reviews/Basics%20of%20Kraft%20Pulping.pdf

http://biorefinery.utk.edu/pdf/Molecular_Weight_Distribution_of_Lignin.pdf